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A COURSE

OF

PRACTICAL INSTRUCTION

IN

BOTANY

BY

F. O. BOWER, D.Sc., F.L.S.

REGIUS PROFESSOR OF BOTANY IN THE UNIVERSITY OF GLASGOW

THIRD EDITION

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PREFACE

THIS *Course of Practical Instruction in Botany* having now passed through two editions, it will be unnecessary again to state the history of its origin; this has been sufficiently set forth in the Preface to the earlier Editions.

The book now stands for the first time as a connected whole, with continuous pagination: as compared with the previous editions of the two parts, hitherto separate, the present issue shows but slight changes, excepting as regards arrangement. The chapter on Microchemical Reactions (Chapter III.) has been greatly curtailed, and the specific reactions of the less common substances in the plant-body have been introduced in the text, at various points where such substances may be met with in the study of the types.

In dealing with the Angiosperms the description of work to be done on the seed and fruit, and on germination has been placed after that on the ovule and fertilisation; this is its reasonable position. The

terminology of the tissues of the root has been revised in accordance with recent views.

A chapter has been devoted to the Characeæ, which were entirely omitted in previous editions; various subsidiary types have been introduced here and there in the text in small print, with a view to extending the course beyond the dangerously narrow limits of pure type-teaching. These and other alterations have been made in response to the remarks and suggestions of critics and friends, to whom I take this opportunity of expressing my obligation.

F. O. Bower.

Glasgow, *April* 1891.

TABLE OF CONTENTS

PAGE

List of Apparatus 1

INTRODUCTORY CHAPTERS.

I. { A.—Making Preparations 4
B.—Adjustment of the Microscope 17

II.—Practical Exercises involving Simple Methods . 24

III.—Common Micro-Chemical Reactions 35

PRACTICAL DIRECTIONS FOR THE STUDY OF TYPES

PHANEROGAMÆ.

I. ANGIOSPERMS.

† VEGETATIVE ORGANS.

A.—DICOTYLEDONS.

PAGE

STEM—*HERBACEOUS TYPE.*
Mature 55
Young 76
Apical Bud 79
Node 86
ARBOREOUS TYPE 88
AQUATIC TYPE 110
Sieve Tubes 113
Laticiferous Tissues 118
LEAF—*Bifacial Type.*
Petiole 122
Lamina 124
Isobilateral Type 134
Centric Type 136
Aquatic Type 138
LEAF-SCARS, AND FALL OF LEAF 143
HAIRS AND EMERGENCES 145
ROOT—*Herbaceous Type* 148
Ligneous Type 153
Apex 157

B.—MONOCOTYLEDONS.

STEM—*HERBACEOUS TYPE* 161
BULBOUS TYPE 167
ARBOREOUS TYPE 169
AQUATIC TYPE 171
LEAF—*Bifacial Type* 172
Isobilateral Type 179
Aquatic Type 180

PHANEROGAMÆ (*continued*).

PAGE
ROOT 180
APEX 183
AËRIAL ROOTS 185

† † REPRODUCTIVE ORGANS.
OBSERVATIONS WITH THE NAKED EYE . . . 187
DEVELOPMENT OF THE FLOWER 194
CALYX AND COROLLA. 197
THE STAMEN 200
CARPEL AND OVULES 204
FERTILIZATION 206
DEVELOPMENT OF THE EMBRYO.
Dicotyledon 209
Monocotyledon 211
DEVELOPMENT OF ENDOSPERM 212
MATURE SEED AND EMBRYO.
Dicotyledons 215
Monocotyledons 218
RESERVE AND TRANSITORY MATERIALS IN SEEDS, TUBERS, &C. 220
GERMINATION.
Dicotyledons 225
Monocotyledons 227

II. GYMNOSPERMS.
† VEGETATIVE ORGANS 229
STEM 232
LEAF 244
ROOT 247
† † REPRODUCTIVE ORGANS 252
RIPE SEED AND GERMINATION 258

PTERIDOPHYTA.

A.—LYCOPODINEÆ.

I. SELAGINELLA (Heterosporous Type).
SPOROPHYTE 261
OOPHYTE 272

PTERIDOPHYTA (*continued*).

II. LYCOPODIUM (Homosporous Type).

PAGE

SPOROPHYTE 275

B.—FILICINEÆ.

I. ASPIDIUM (Homosporous Type).

MATURE SPOROPHYTE.

EXTERNAL CHARACTERS 287

ANATOMICAL CHARACTERS TO BE OBSERVED WITH THE NAKED EYE 289

MICROSCOPIC OBSERVATIONS—

Stem 292

Root 303

Leaf 307

Sporangia 309

OOPHYTE 311

YOUNG SPOROPHYTE 317

II. PILULARIA (Heterosporous Type) 319

C.—EQUISETINEÆ.

EQUISETUM.

SPOROPHYTE 325

OOPHYTE. 340

BRYOPHYTA.

A.—MUSCI.

POLYTRICHUM.

GENERAL EXTERNAL CHARACTERS 341

MICROSCOPIC INVESTIGATION—

OOPHYTE 342

SPOROPHYTE 350

SPHAGNUM 357

B.—HEPATICÆ.

MARCHANTIA.

GENERAL EXTERNAL CHARACTERS 360

MICROSCOPIC OBSERVATIONS 363

SPOROPHYTE 376

THALLOPHYTA.

A.—ALGÆ.

FLORIDEÆ.

PAGE

POLYSIPHONIA 379

PHÆOPHYCEÆ.

FUCUS 391

LAMINARIA 406

CHARACEÆ 409

CONFERVOIDEÆ.

COLEOCHÆTE 419

ÆDOGONIUM 421

ULOTHRIX 424

CLADOPHORA 427

SIPHONEÆ.

VAUCHERIA 429

VOLVOCINEÆ.

VOLVOX 437

PLEUROCOCCUS 439

HYDRODICTYON 441

CONJUGATÆ.

SPIROGYRA 442

DESMIDS 447

DIATOMS 449

CYANOPHYCEÆ.

NOSTOC 451

OSCILLATORIA 452

GLÆOCAPSA 452

B.—FUNGI.

BASIDIOMYCETES.

AGARICUS 453

ÆCIDIOMYCETES.

PUCCINIA 462

THALLOPHYTA (*continued*).

ASCOMYCETES.

PAGE

PEZIZA 470
PARMELIA 473
CLAVICEPS 481
EUROTIUM 484

PERONOSPOREÆ.

PYTHIUM 491
CYSTOPUS 495

MUCORINEÆ.

MUCOR 497
SPORODINIA 500

APPENDIX A.—*List of Reagents, their Preparation, and Uses* . . 503

APPENDIX B.—*List of the Reactions of Bodies commonly found composing the Tissues of Plants* 518

APPENDIX C.—*List of the Specimens mentioned in the Book: the sources from which they may be obtained, and their preparation* 523

INDEX 537

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PRACTICAL BOTANY

The following is a list of apparatus required for ordinary work in the botanical laboratory. The articles marked with an asterisk (*) are absolutely essential to successful work

1. A pair of fine **scissors** with sharp points.
2. Fine-pointed **forceps.**

*3. One or more **good razors** (see p. 8), and a strop and hone for sharpening them.

4. **Scalpels** of various sizes : a fine eye-scalpel with a long narrow blade will be found to be very useful.
5. A **section-lifter.**

*6. Mounted **needles.**

7. Several fine **camels'-hair brushes.**

*3. **Watch-glasses** of various sizes, flattened at the middle of the convex side so as to stand steadily.

*9. Glass or porcelain **ointment pots,** with lids.

10. **Test-tubes** and **beakers.**
11. **A spirit-lamp.**
12. A **black enamelled tile** for mounting on.

*13. **Glass slides,** with ground edges (3 in. × 1 in.).

*14. Thin **cover-glasses,** square, or circular ($\frac{7}{8}$ in. diameter).

*15. **Blotting-paper,** cut or torn into small pieces.

*16. **Drawing-paper** or **card,** with a hard smooth surface, or a note-book of such paper, without lines.

*17. Hard **pencils** (H. or H.H.H.) and india-rubber.

*18. Gummed **labels** (1 in. × $\frac{3}{4}$ in.) for naming slides.

*19. A coarse **duster,** and a finer **cloth,** *e.g.* an old pocket-handkerchief.

20. A **rack** for keeping slides temporarily, and a **bell-glass** to cover it.

*21. A **simple lens.**

*22. A **compound microscope**. This should be one of the smaller stands with a short tube, *e.g.* Hartnack No. III. A, or Zeiss No. V., VI., or VII. : similar stands of varying merit are to be obtained from most of the English makers. The microscope should be provided with—

*i. High and low **eye-pieces**: the longer is the lower power, the shorter the higher.

*ii. Two **objectives,** the lower power (Zeiss A, or Hartnack No. 3) of about **1 inch** focal length. The higher (Zeiss D, or Hartnack No. 7) about **one-sixth inch or one-eighth inch** focal length.

*iii. A **micrometer,** either adapted to the eye-piece, or a stage micrometer.

iv. A **nose-piece** to carry two, or, if necessary, more objectives : its use will save much time.

v. A **camera lucida** for drawing.

vi. An **erecting eye-piece** is also a useful adjunct, when dissection is to be carried on under the microscope.

*23. A rack or tray to hold small glass-stoppered bottles containing **reagents** : the following are the reagents which are in most constant use—

a.* **Weak glycerine, *i.e.* Price's pure glycerine diluted with an equal volume of distilled water.

b.* **Caustic potash: make a 2 per cent. solution of the solid sticks of caustic potash in distilled water, and filter.

c.* **Acetic acid : one volume of glacial acetic acid is to be diluted with 99 volumes of distilled water.

d.* **Iodine solution : this may be obtained by diluting the

liquor iodi of the Pharmacopœia ; or as follows : dissolve a small quantity of potassium iodide in distilled water, and add crystals of iodine : if the solution be too deeply coloured it may be diluted with distilled water to the colour of brown sherry.

*e. **Chlor-zinc-iodine** (Schulze's solution) may be purchased ready prepared from the dealers in microchemical reagents ; or it may be prepared as follows :—

(1) Dissolve 110 grms. of zinc in 300 c.c. of pur hydrochloric acid, and evaporate to 150 c.c. (sp. gr. about 1·8).

(2) Dissolve 12 grms. of KI in as little water as possible : add 0·15 grm. of iodine.

(3) Mix (1) and (2), and filter, if necessary, through asbestos. The solution should have a dark sherry-brown colour.

*f. Solution of **aniline chloride**: a saturated solution is made in distilled water, filtered, and a few drops of hydrochloric acid added so that it may give a distinctly acid reaction. The solution should be colourless.

*g. A solution of **common salt** : a 5 per cent. solution, *i.e.* 5 grms. of salt to 100 c.c. distilled water.

Many other reagents besides these will be required for the work described below : also substances for permanent mounting and sealing up of slides : their preparation and uses are detailed in the Appendix A.

Care should be taken in the preparation of the reagents : they must be kept pure, and should be renewed occasionally. **Glass rods** with rounded ends are to be used for removing drops of the reagents from the bottles to the slide, and *the rod should always be cleaned before dipping it into a reagent-bottle.*

*24. Two **wash-bottles** such as are in ordinary use in a chemical laboratory : the one should contain *alcohol* (methylated), the other *distilled water.*

1

A.—*Making Preparations*

I. **Preservation of Material.**—In many cases it is possible, and even preferable, to use fresh material, but it is often convenient to keep it for a time, since many of the specimens required are only to be obtained at certain seasons of the year: the best liquid for this purpose is ordinary methylated alcohol, in such quantity as completely to cover the material. It must be remembered that this will extract the green colouring matter (chlorophyll) from the material immersed in it, as well as resin and other substances.

II. **Hardening.**—It is not necessary, for the general study of the histology of the mature parts of plants, to harden them, for the tissues are usually sufficiently firm to admit of their being cut satisfactorily. In the case of young, or of exclusively parenchymatous tissues especially those of non-vascular plants, it is necessary, to harden them, and for this purpose alcohol may be used.

When it is desired to study the structure of the protoplasm, and of the nucleus, special methods must be employed for hardening them, or rather for fixing

them as nearly as possible in the condition in which they are during life. For this purpose one or other of the fluids mentioned below may be used. Care must be taken that the objects shall be of small size, that the quantity of hardening fluid is large relatively to the bulk of the object, and that the fluid has ready access to all parts of it. Large objects should be cut up into pieces of moderate size, so that the reagent may readily gain access to all parts of the tissue.

The following are the best fluids for this purpose :—

1. Absolute alcohol or methylated spirit.
2. Picric acid (saturated solution in water).
3. Chromic acid (0·1—0·5 per cent. solution in water).
4. Osmic acid (·1—1· per cent. solution in water).

These reagents are only to be applied to fresh material.

When absolute alcohol is used, the object may be kept in it for an indefinite period. Such treatment generally makes the object brittle; this may be remedied when the object is to be mounted in glycerine by placing it, for at least twenty-four hours before it is to be cut, in a mixture of glycerine and absolute alcohol in equal parts, leaving it exposed to the air so that the alcohol may gradually evaporate. The glycerine slowly saturates the object and restores its toughness. This can only be done when the sections are to be mounted in glycerine.

When picric or chromic acid is used, the object should be immersed in it until each part of it is thoroughly permeated by the reagent; the length of time required for this varies with different material, and in the case

of chromic acid, with the strength of the solution used, from a few minutes to twenty-four hours or more. The objects must then be washed thoroughly with water: they are then to be placed in dilute methylated spirit (50 per cent.), subsequently in stronger spirit (70 per cent.), and finally in absolute alcohol or strong methylated spirit, which must be changed so long as any colour is still extracted from the objects. They may be preserved in this for future use.

When osmic acid is used, the fixing effect is produced much more rapidly; in the case of simple structures, such as unicellular or filamentous Algæ, a few minutes (5—15) generally suffices; in the case of more complex structures, such as ovules, sporangia growing points, &c., the object may be left in the acid till it looks black on the exterior: it must be then well washed with dilute alcohol (50 per cent.), and left in it for some time, and be then removed to 70 per cent. The sections are best mounted in dilute glycerine. In some cases osmic acid produces an excessive blackening of the cells, which can be removed by treatment with chlorine-water.

Of the hardening reagents above mentioned absolute alcohol, methylated spirit, and picric or chromic acids are those most generally used.

The following is a useful method for preparing sea-weeds: to a quantity of saturated solution of picric acid in sea-water add three or four times its volume of sea-water, and treat the tissue with it for ¼ hr. to 2 hrs.: wash well with sea-water so as to remove the picric acid, and then treat successively with 30, 50, 70, and 90 per cent. alcohol.

It is advisable in cases in which the cell-walls tend to swell up

excessively (as in many Algæ) to use solutions of picric, chromic, or osmic acids, to which an equal volume of absolute alcohol has been added.

III. **Cutting Sections.**—In order to investigate the structure of the tissues of a plant or member, it is usually necessary to cut sections, *i.e.* thin slices, in various directions. To make a complete study of a solid mass of tissue, sections must be cut in three different planes at right angles to one another. Taking the case of a cylindrical stem, the best way to study its structure would be to cut—

(i.) **Transverse sections,** in planes at right angles to the organic axis.

(ii.) **Radial longitudinal sections,** in longitudinal planes including the organic axis.

(iii.) **Tangential longitudinal sections,** in longitudinal planes which do not include the organic axis.

This may be illustrated by a diagram (Fig. 1), which may be taken to represent the transversely cut end of a cylindrical stem, the tissues being arranged with reference to a central point (E): **transverse** sections are those which are in transverse planes, parallel to the plane of the paper in Fig. 1. The line including the central points of successive imaginary transverse sections is the organic axis.

Radial and tangential sections are both in planes vertical to that of the paper in Fig. 1: a **radial** section (A E B) includes the organic axis (E), and a slice of tissue thus cut when examined from a direction indicated by either of the arrows (*x*) will show in surface view those cell-walls which run radially: a **tangential** section (C D) does not include the organic axis (E), and

such sections when examined from a direction indicated by the arrows (*y y*) will show the tangential walls in surface view, while the radial walls, previously seen in surface view would present their cut edges to the observer.

In the case of tangential sections only the central part of the section (*i.e.* the part near to *y y*) is to be

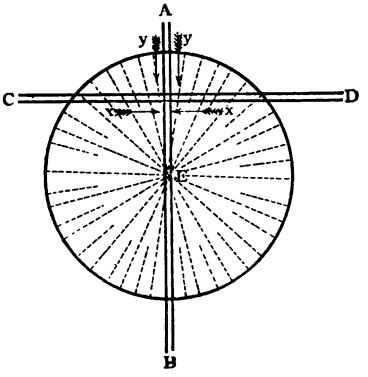

FIG. 1.

examined, for obviously in the more lateral parts of the section (C D) the radial lines are cut not vertically but obliquely.

In all cases the sections must be cut accurately in the plane intended: if the sections be cut obliquely the difficulty of understanding the structure will in almost every case be enormously increased.

A **razor** of good quality is the best cutting instrument: there is some variety of opinion as to the best form of blade; some prefer a hollow-ground razor, which, though well suited for cutting small sections, will not

serve for sections of large area; for this work a razor with one flat side is recommended. For general use, not only in cutting small objects and soft tissues, but for the every-day work of the laboratory, an ordinary, very slightly hollow-ground razor, of good quality will be found the most useful. The razor should be stropped to a smooth edge, and the blade should be carefully protected when not in use: it should never be left open on the work-table, and the blade should always be cleaned after use, since the acid juices of plants are apt to corrode it. It will be found convenient to have a glass of water (or weak spirit when resinous tissues are being cut) on the work-table, into which the blade of the razor may be plunged at once after use; this will prevent immediate corrosion.

The success of work in the laboratory depends very greatly on due care in the direction of section, and on the condition of the edge of the razor.

Almost all the sections required in the succeeding pages of this book can be made by hand: elementary students are advised to avoid the use of a microtome, which is for their work a useless and expensive incumbrance; they should rather cultivate that small amount of manual dexterity which will suffice for the successful preparation of almost all the objects to be described below. When however a series of successive sections of an object is required, a microtome may be used, care being taken to keep the sections in their proper order, and the right way up.

For advanced students a microtome may be found a convenience, though it is not actually necessary for any of the work detailed in this book. This being so, no description will be given

of the special methods of preparation of objects for cutting by the microtome, nor of the different forms of microtome, or the way in which the microtome is to be used.

When cutting sections the razor is to be opened so that the blade is in a line with the handle: the object to be cut may be held in the thumb and first finger of the left hand, while the razor is grasped firmly by the four fingers of the right: it may be found convenient to rest the thumb of the right hand on that of the left so as to regulate the movements of the right hand. The edge of the razor is not to be rudely forced through the tissues of the specimen, but a sliding cut is to be made, thus using a considerable length of the edge of the razor: in this way a smoother surface of section is obtained, and the tissues are not displaced as they otherwise might be.

Care must be taken to keep the object and the razor wet during the process of cutting, in order to avoid the entrance of air into the tissue, and to prevent adhesion of the section to the razor. When fresh material is cut, water or very dilute alcohol may be used for this purpose, but if material which has been hardened is cut, it is advisable to use alcohol of the same strength as that in which the material has been preserved.

IV. **Embedding.**—The objects are frequently so large that they may be held in the hand whilst they are being cut. If they are too small for this, it is convenient to embed them in some substance.

The simplest method is to fix the object into a slit in a piece of pith. Dried elder-pith is the best, and it may be bought ready prepared from the dealers.

When the sections are to be made with a microtome

or when the object to be cut is small, or easily damaged, it is more convenient to embed in some easily fusible substance: by this means also the form of the object is less likely to be distorted in the process of cutting. Various substances, or mixtures of substances, are used for this purpose, of which the following is perhaps the best :—

Solid paraffin (melting-point about 58° C.): 2 parts.
Vaseline: 1 part.

These must be melted together and well stirred. The resulting substance is sufficiently transparent to enable the exact position of the object to be ascertained; it is easy to cut, and it is readily soluble in carbolic acid and turpentine. The relative proportions of paraffin and vaseline may be varied somewhat to suit the object; a softer mixture is produced by increasing the proportion of vaseline. Samples of paraffin which vary in hardness and melting point may be obtained from the dealers, the softer paraffin with low melting point will be the most useful.

The ordinary method of embedding is to make a cavity in a piece of the substance sufficiently large to contain the object, which, if fresh, must have been previously washed with alcohol to remove all traces of water from its surface. If the object had been previously preserved in alcohol, all superfluous fluid must be removed from the surface with blotting-paper, but care must be taken that the spirit which permeates the tissue shall not evaporate. The object is then placed in the cavity, and without unnecessary delay a small quantity of the embedding substance, melted over a

spirit-lamp in a small tinned iron spoon, is poured into the cavity so as to surround and cover the object.

If the object be small it will be found convenient to heat one end of a thick copper or platinum wire, and with it melt a small cavity, in which the object may be placed in such position as is found convenient.

The sections must not be made until the paraffin is quite cold, and firmly set.

It is important to keep the embedded objects wet with alcohol during the process of cutting, in order to prevent the drying-up of the object, and its consequent contraction away from the substance in which it is embedded.

Another method of embedding is to moisten the object in water, and then suspend it by means of a thread in some white of egg, which has been previously well shaken up, and then strained through muslin. The white of egg should be in an evaporating dish. The object should be left thus suspended for some hours, so that the white of egg may come into close contact with all parts of it. Heat is then applied by means of a water-bath, and the white of egg coagulates. The part surrounding the object is now cut out and hardened in alcohol for some days. This method is useful for making sections of buds and flowers.

A third method of embedding may be employed when it is desired to obtain sections of very small objects, such as spores, pollen-grains, &c. A thick layer of strong clean gum is laid on the flat surface of a piece of pith; this is allowed to become nearly dry; and then the pollen-grains or spores are dusted on to it; they are then covered with another thick layer of gum, and the whole is allowed to dry. Sections are now made of the dried gum, and on their being placed in water or weak glycerine, the gum is dissolved, and the sections of the pollen-grains or spores are set free.

The sections when cut should be removed at once to a watch-glass containing alcohol or water, by means of a camels'-hair brush, or a jet of alcohol or water from a wash-bottle: the useless thicker sections may then be removed, and the thinnest ones selected for observation.

V. **Mounting Objects.**—Various specimens, whether sections or objects which may be examined whole, require very varied treatment, and the common methods in ordinary use will be described below, and illustrated by experimental exercises (see pp. 25, &c.); meanwhile a few practical suggestions will be given which are to be observed in all cases, whatever the special method of treatment may be.

1. *Study to avoid all unnecessary manipulation of specimens; never apply a reagent at haphazard, but only when you have a definite purpose for doing so.*

These rules apply specially to staining reagents, which should only be used when their assistance is actually required: the primary end of the anatomical investigations detailed below is not to prepare a number of objects pleasing to the uneducated eye, but to gain a knowledge of the structure of the plant-body as it is in the living state, and this end may as a rule be best attained by the simplest methods.

2. See that the glass slide and the cover-glass are *perfectly clean* and dry, and show a bright polished surface before using them; they should be cleaned immediately before use, and, after cleaning, their surfaces should not be touched with the fingers, nor should the cover-glass be laid flat on the table, but tilted on its edge.

New cover-glasses are sometimes difficult to clean: heating them with very dilute sulphuric acid, and subsequently washing with water, will usually be found successful. Old slides may often be cleaned with ease with water only: if this be unsuccessful try washing with turpentine, or heating in a potash solution, or if that does not do, in dilute sulphuric acid.

3. In mounting, whatever the fluid may be, take *only so small a drop of it as shall just suffice to fill the space between the slide and the cover-glass, and extend to the margin of the cover:* judgment as to the quantity necessary can only be acquired by practice. If too much fluid has been used the excess must be soaked up with slips of blotting-paper, or filter paper.

4. *The practice of scrupulous cleanliness cannot be too strongly impressed upon students as the basis of all successful work with the microscope,* and it is in the use of fluid reagents that the greatest care is necessary; if too large a quantity be used it is apt to extend to the lower surface of the slide, and so to the stage of the microscope; or to be smeared over the upper side of the cover-slip, and may then gain access even to the objective; *it is absolutely necessary that both the front lens of the objective, and the upper side of the cover-slip be perfectly clean and dry, also the lower surface of the slide and the stage of the microscope.*

5. Having taken a sufficiently small drop of the mounting medium, and having placed the object in it, bring down the cover-glass *obliquely upon the drop so that one edge of it is first wetted by the medium,* then let down the slip gently, so as to allow the medium time to spread out under the cover-slip; this may be done either by holding the cover-slip in a pair of clean

forceps, or hold the slip by its edges in an oblique position in the finger and thumb of the left hand, while it is supported by a clean needle held in the right; then, the lower side being wetted with the medium, gradually withdraw the needle and thus gently lower the slip. It would be well at first to practise thus lowering the cover-glass over a drop of water, so as to acquire judgment of the quantity of fluid required, and skill in avoiding the inclosure of air-bubbles.

6. One great purpose of the above directions is to avoid the presence of **bubbles of air** in the medium surrounding the object; their presence is one of the great difficulties of the beginner, who is therefore advised, in his own interest, to follow carefully the directions above given. In some specimens, especially when fresh, air-bubbles will be found entangled in the tissues, or attached to the outside: a good method for avoiding them in mounting fresh material is to moisten with alcohol (weak alcohol will do) for a few seconds before mounting: by this means the surface of the object will be more thoroughly wetted than would otherwise be the case. Obstinate bubbles may be expelled by heating over a spirit-lamp; but as many objects will not stand such rough treatment, a better method is to exhaust them under the receiver of an air-pump.

7. After an object has been mounted it is often necessary to apply to it certain staining, or micro-chemical reagents: this may frequently be done, without removing the object from the slide, by **irrigation**: successive drops of the reagent are placed on the slide, close to one edge of the cover-slip (*special are being taken that the fluid does not spread to the upper*

side of the cover), while a small piece of blotting-paper is pushed up to the opposite edge of the cover-slip, so that, when it comes in contact with the medium in which the object is mounted, it will soak it up: the space thus vacated by the medium is taken by the reagent, and if the latter be supplied in sufficient quantity a stream of it will pass under the cover-slip and bathe the object. It is obvious that for such a treatment to be successful the medium and the reagent must be fluids which will mix. Students are warned against too readily accepting negative evidence as the result of observations by irrigation: the reagent may frequently pass under the cover-slip without permeating the object, or the edges only of a section may be affected: in order to insure the object being bathed by the reagent, it is well to raise the cover-glass gently with a needle, or even to raise the section itself slightly with the point of a needle.

8. *Never use more than one cover-slip on a single slide,* though several objects may, if small enough, be covered by one slip: *the cover-slip should be as nearly as possible in the middle of the slide.*

9. *Pressure should never be laid on the cover-slip,* except in certain special cases: a bad section will not be improved by being squeezed flat, while a good section may be easily rendered worthless by such treatment.

10. Before putting a slide aside for subsequent observation *be sure that the medium used is one which will not evaporate:* alcohol, water, and solutions in water, such as iodine solution, aniline sulphate, salt solution, are all liable to evaporation, while chlor-zinc-iodine and glycerine are not.

11. Before putting a slide aside *be careful to label it,* writing at once on an adhesive label the *name of the plant, part, direction of section, and medium in which it is mounted.*

B.—*Adjustment of the Microscope for Work.*

Before beginning work it should be ascertained *that the microscope is in good working order:* if it be a stand without rackwork, see that the stage of the microscope is clean, and that the tube moves easily and smoothly; if not, take out the tube, and rub it with a clean cloth; if still stiff, apply a very little mineral oil or vaseline, and rub with a clean cloth till there is no appearance of oil on the tube: it should then work freely. Nothing causes a greater strain on a microscope than neglect of this simple point.

Next *see that the lenses are clean;* to this end first dust the mirror, and adjust it so as to reflect light through the instrument: insert first one eye-piece, then the other: rotate each eye-piece, and if any specks be seen to rotate with it they are on the lenses of the eye-piece, and must be removed with a soft chamois leather, or a fine linen or silk cloth (old pocket-handkerchief). Carefully examine the front lens of each objective, and if any dirt be seen wipe the lens *gently* with a chamois leather, or fine linen or silk cloth. Glycerine is apt to gain access to the objective in careless hands; when this is the case the lens is to be washed with a jet of distilled water, and carefully dried. Since the lenses are often fixed with balsam, great care must be taken that the lens

should not be smeared with Canada balsam or Dammar: when this has happened the lens should be gently rubbed with a cloth wetted with a very little benzol, or alcohol. In all cases the cleaning of lenses should be carried out as gently as possible, to avoid destroying their polish.

The best light for microscopic work is that reflected from white clouds in a northern sky, and a window with a northern aspect should be selected. *Never use direct sunlight, and avoid using artificial light.* If the only available room has a south aspect, a white blind is to be used, so as to cut off direct sunlight, or a piece of white card may be fitted to the surface of the mirror, so as to act as a less perfect reflector.

The *body of the microscope should be vertical;* with the short microscopes now in use, the oblique position is quite unnecessary, and very inconvenient when mounting in fluid media, or irrigating with fluid reagents.

Always *examine an object with a low power first,* and afterwards, if necessary, with a higher power. It is a general principle of microscopic practice that *observations should be made with the lowest possible power sufficient for distinct vision. Never use the high power unless the object be covered with a cover-slip.*

When a *low power* is used a *larger hole of the diaphragm* below the stage is to be placed opposite the aperture in the stage; when a *high power* is used a *smaller hole of the diaphragm* is necessary, otherwise the definition will not be satisfactory.

Some difficulty will be felt at first in *finding the focus.* There are two adjustments of focus—the coarse and the fine: the latter is never to be used until the focus is approximately found with the coarse adjustment.

The coarse adjustment is effected by a sliding tube in the smaller microscopes in general use by students: having drawn this tube out, screw on the low power objective (the one with the larger front lens, focal length about 1 inch from object), then replace the tube so that the objective is about $1\frac{1}{2}$ inches from the stage, and having adjusted the mirror so as to illuminate the whole field, place some object on a slide at the centre of the stage; hold the slide with the thumb and forefinger of the left hand, while the upper end of the tube is grasped with the right: then slide the tube gradually downwards with a spiral movement, until the object comes dimly into view: then begin to use the *fine adjustment*, which is worked by a screw with a milled head, the position of which varies in different instruments: this head is to be turned so as to lower the tube, when the object will become clearer, and ultimately in perfect focus. The focus is to be found in the same way with the high power, but in this case greater care is necessary, since when in focus the objective is nearer to the object: in careless hands the position of focus is apt to be overstepped, and the objective advanced so as to touch, or even crush, the object: *this is to be carefully avoided*, as it not only damages the object, but may also ruin the objective.

When the focus has been found, the fine adjustment is to be worked constantly up and down by the right hand during observation; by this means a series of optical sections of the object is brought successively into view, and in this way the observer builds up mentally a conception of the object *as a solid body*. In so far as an objective lends itself to this it is said to possess

good *penetration*. Meanwhile the forefinger and thumb of the left hand will be at liberty to move the slide on the stage so as to bring into the field of view different parts of the preparation.

Observers should accustom themselves to using both eyes indifferently, and when one eye is being used for observation, the other should be kept open: a little practice will soon overcome any difficulty which may be at first found in doing this.

Care is necessary in removing the slide from the stage, especially when the high power has been used: in this case the tube should first be raised so as to remove the objective from close proximity to the stage, and the *slide should then be slipped off the stage*, not lifted off. Want of attention to these points is apt to result in smearing the objective with glycerine, or other media.

Drawing from the Microscope.—Nothing compels attention to details of an object so successfully as drawing it; while, as it is impossible to make a drawing of an ill-prepared object, the intention to make a drawing will have its effect upon the care devoted to preparation and mounting. *It should be a rule for students to draw every object they observe*, not merely for the sake of the drawings as memoranda, but in order to acquire a habit of close observation.

For drawing, a hard pencil (H.H.H.) is recommended, and it must be cut to a fine point: paper with a hard smooth surface is to be used, or better, a thin drawing card or Bristol board with a hard surface. A decisive style of drawing should be adopted, in which every line is clear, and conveys its own meaning.

For ordinary purposes a freehand drawing will suffice, the scale being as nearly as possible that of the object as it appears under the microscope, or larger, if the object be a complicated one: whatever the scale, the *proportion* of the several parts is to be scrupulously followed. Coloured chalks, or better, light washes of water-colour, may be used for distinguishing tissues of different character, and in making a series of drawings the same colours should be assigned to corresponding tissues throughout the series.

When drawing cell-walls of appreciable thickness, they should be indicated by a double line; solid bodies should be shaded so as to give the idea of light coming from one side, and in a series of drawings the side selected should be maintained throughout.

But in case of complicated networks of tissue, or where exactness of delineation, proportion, and scale, are required, drawings should be made by the help of the *camera lucida*. A most convenient type of camera is that of Nachet of Paris; and it may be obtained from Swift and other makers in a form adapted to English microscopes. It is convenient to have it fitted with a spring clip, by which it is attached to the upper extremity of the tube of the microscope, while the camera itself is connected with the clip by a hinged arm, so that it can be adjusted over the eye-piece when in use, or when not wanted, it can be turned to one side without being actually removed.

When an object is to be drawn, it must be placed in the middle of the field of the microscope, which is to be kept as usual in the vertical position; a sheet of paper or card is placed on the table to the right of the microscope, and the camera adjusted over the eye-piece: on looking through the camera the object will still be seen, and the movable prism is then to be rotated on its axis till the sheet of paper comes also into view: the object will then be seen projected on the sheet of paper, and its outline may be traced

off. With regard to this method of drawing the following remarks are to be noted :—

i. The adjustment of light is sometimes difficult, so that the object and the point of the pencil may be seen simultaneously with equal clearness : if the illumination of the field be too faint, the paper is to be darkened by a screen placed between it and the window, while if the field be too bright the tinted glass which is usually supplied with the camera may be adjusted so as to cut off part of the light which comes through the instrument.

ii. This method of drawing, though convenient, is open to objection on the ground of distortion of the image : to avoid this, an inclined desk may be used, so adjusted that the plane of the paper may be perpendicular to a line drawn from the camera to the centre of the image projected on the paper. But where the area of the object is not great the distortion may be neglected, *provided the drawing be made as near as possible to the foot of the microscope.*

iii. In drawing with the camera it will be found convenient first to take a trace in very faint lines, and then, removing the camera, to finish the drawing, and put in the details with a free hand, always maintaining a close observation of the object.

iv. An exact measure of the scale of the drawing under any given power may be obtained by the following simple method. Take a *stage micrometer* with parallel lines ruled on it at distances of $\frac{1}{1000}$ths of an inch : draw these lines by help of the camera under the magnifying power which it is desired to measure, and then with an ordinary rule ascertain the distance between the lines drawn : divide this into $\frac{1}{1000}$ths of an inch, and the result is the scale of enlargement of the drawing.

v. On every drawing made with the camera *the magnifying power should be noted at once,* as well as the number or letter of the eye-piece and objective.

Measurement of Objects.—Measurements may be most readily made by means of an **eye-piece micrometer,** which is a glass slip, fitted into the eye-piece, and having a scale engraved upon it. The value of the

divisions of this scale varies with the combination of lenses used; accordingly, before the micrometer can be employed in the actual measurement of objects, the value of the divisions must be determined for each combination, and a table of the results should be kept for reference in the case of the microscope. To determine the value of divisions of the scale under a given combination of glasses, a stage micrometer, having lines drawn to $\frac{1}{1000}$ths of an inch apart, should be placed on the stage, and focussed under the objective and eye-piece whose magnifying power it is desired to measure: the relation of the divisions of the stage micrometer (these intervals being of known value) to those of the eye-piece micrometer is then to be noted. Suppose that the interval between two lines of the stage micrometer covers the intervals between *six* lines of the eye-piece micrometer, the former being $\frac{1}{1000}$th of an inch apart, the interval between two lines of the latter (with that combination of lenses) will correspond to $\frac{1}{5000}$th of an inch, and the *linear* measurement of any object which fills such an interval under that combination of lenses will be $\frac{1}{5000}$th of an inch. It is usual to state the size of objects seen under the microscope according to the linear measurement of the diameter. The simpler method of measurement by laying the stage micrometer inverted on the slide carrying the object to be measured, though direct, is open to many objections, and can at best only be used with low powers.

II

PRACTICAL EXERCISES ON THE STRUCTURE OF THE VEGETABLE CELL INVOLVING SIMPLE METHODS OF PREPARATION

Before entering upon the work described below, the preceding pages should be carefully read through, otherwise the beginner will be apt to make serious mistakes in manipulation.

NOTE.—The method of mounting fresh material in water will be used throughout this chapter: it has the following advantages:—

1. It is the simplest possible.

2. The cells are seen unaltered, *i.e.* in the living state, and it is thus specially suitable for observations on fresh material.

3. When thus mounted the effect upon the living cell of any reagent soluble in water may be observed by irrigation: thus it is the natural starting-point for the study of the micro-chemical reactions of the living cell.

It is however open to objection on the following grounds:—

1. The slides thus prepared cannot be kept, since the water would evaporate.

2. The refractive index of water being relatively low, the objects do not appear so transparent as in more highly refractive media.

3. Bubbles of air are very apt to be included with the object.

I. Take the most mealy-fleshed Apple that is to be had (a mellow American Baldwin pippin will do well), split it in half, and with a scalpel remove a *very small* portion of the pulp near to the core: place it in a single drop of water on a glass slide, and tease it out gently with needles, so as to separate its constituent parts: cover it with a cover-slip (p. 14), and examine under a low power (1 inch): focus carefully (p. 18), and observe—

1. Numerous, more or less irregularly oval, colourless sacs, lying sometimes isolated, sometimes associated together in irregular groups: these are the constituent **cells** of which the pulp is composed: having been exposed to no treatment which would injure them beyond the mechanical disturbance of teasing, most of them will be *alive*, and will maintain their oval form.

Select one such, adjust the slide so that the cell shall be in the middle of the field, put on the high power ($\frac{1}{6}$ or $\frac{1}{8}$ inch: see pp. 18, 19), focus carefully, examine it in detail: note—

2. That it is limited by a thin, smooth, continuous membrane—the **cell-wall.**

3. That in close apposition to this internally is a film of transparent granular substance: this is the

protoplasm (**"primordial utricle"** of the older botanists).

4. A large internal cavity bounded by the protoplasm: this is the **vacuole,** or cell-cavity.

The distinction between the parts of the cell may be brought out more clearly by means of the following reagents:—

a. Irrigate (p. 15) continuously for some minutes with a $2\frac{1}{2}$ per cent. solution of *common salt* (that is, the 5 per cent. solution diluted to double its volume): the cell-wall will retain its original position and appearance, but the film of protoplasm will be seen to contract and gradually to separate from the cell-wall; the separation usually extends over only a part of the surface, but it is sufficient to allow the cell-wall and protoplasm to be easily distinguished.

b. Treat a freshly prepared specimen with *iodine solution:* the cell-wall is not appreciably stained, but the protoplasm stains a more or less deep brown. A small oval body, which stains a deeper brown than the rest of the protoplasm, may often be seen included in it: this is the **nucleus.**

c. Treat another fresh specimen with *glycerine,* and watch the effect: many of the cells will lose their oval form, and collapse, the cell-wall being thrown into irregular folds. From this it will be at once seen that if specimens of a fresh tissue be placed directly in glycerine the cells are liable to change of form, which is owing to the sudden extraction of water from them: care should therefore be taken in the treatment of fresh material with glycerine, otherwise good specimens may be readily spoiled.

d. Treat a fresh preparation of the Apple pulp with *chlor-zinc-iodine* (Schulze's solution), and examine it under a high power: observe—

i. That the protoplasm is stained brown as with the iodine solution above used.

ii. That the cell-wall is stained violet.

It is to be noted that the stain does not always succeed at once, but time should be allowed for the colour to develop: also that the colouring is not always uniform, so that though the presence of the colour is **characteristic of cellulose,** its absence does not prove that there is no cellulose present: this reaction affords good positive, but not trustworthy negative, evidence.

II. The simple specimen above described, though derived from the complicated mass of tissue of which the Apple consists, will serve to illustrate the appearance of single cells, since these are readily isolated by the process of teasing: the next specimen to be studied will illustrate the aggregation of cells in a linear series, so as to constitute a simple filament.

Mount a *small quantity* of the fresh, filamentous Alga *Spirogyra* in water (this Alga is commonly to be found in summer in stagnant, or slowly flowing, fresh water, as bright green flocculent masses, which are slimy to the touch): examine it first under a low power, and observe on one of the largest specimens—

1. That the cylindrical filament is limited by a definite **cell-wall.**

2. That transverse partitions—**septa**—which are continuous with the limiting cell-wall, divide the filament into a linear series of **cells.**

3. That each cell contains a **protoplasmic body,** the most marked part of which will be one or more **spiral chromatophores,** coloured bright green by chlorophyll.

In order to study the structure of the protoplasmic body in detail, put on a high power, and make the following observations of the filament, which, it is to be remembered, is in the living condition :—

1. Having recognized the smooth colourless **cell-wall,** note that it is closely invested internally by—

2. A continuous film of colourless **protoplasm** (the "primordial utricle"), of which the external layer (**ectoplasm**) is more transparent than the granular internal layer (**endoplasm**).

3. That this protoplasmic film surrounds a large central cavity—the **vacuole**—filled with perfectly transparent **cell-sap.**

4. That in the peripheral film are embedded one or more green spiral bodies (**chromatophores**) of flattened form, and very irregular margin, each including numerous lenticular, highly refractive bodies, the pyrenoids.

5. Focussing carefully downwards into the central cavity, a highly refractive, colourless, lens-shaped body is to be seen suspended in a central position by numerous finely granular protoplasmic threads: this body is the **nucleus.**

a. These several points should be made out in the living cell, without treatment with any reagent; but their observation may be made easier in various ways. Irrigate (p. 15) with *iodine* solution, and observe the following results of that treatment :—

1. The cell-wall will not be appreciably altered or stained.
2. The film of protoplasm (primordial utricle) will have stained a pale yellow or brown, and may often be seen to have separated partially or completely from the cell-wall, from which it may now be readily distinguished.
3. The chromatophores will have assumed an indefinite dusky colour, while the pyrenoids will be a dark purple.
4. The nucleus will have stained a deep yellow or brown, and inclosed within it one or sometimes two **nucleoli** may be recognized by their deeper colour, and high refractive power.

b. Irrigate a fresh specimen with 2½ per cent. solution of *common salt*, and watch the result, which will take some minutes to appear. The protoplasm will gradually separate from the cell-wall, and while the latter retains its form and position, the protoplasm will contract, and rounding itself off, ultimately appear as a more or less irregularly oval or spherical body.

c. Mount a fresh specimen in water, and irrigate with *glycerine;* similar results will be seen to those already observed on cells of the Apple: the cells will collapse, owing to the sudden abstraction of water of the cell-sap.

d. Mount a fresh preparation in water: irrigate with *potash* solution, and observe that the protoplasmic contents swell and lose their definite outline, and the whole becomes more transparent.

III. The above example of a simple Alga shows cells aggregated in a linear series, constituting a filament: the next specimen illustrates the more complicated

arrangement of cells in two dimensions of space, *i.e.* as a flat plate of tissue. Mount a small prothallus of a Fern (or the thin lateral part of a large one) in water, and examine it under a low power. It will be at once obvious that the thin plate of tissue, one layer of cells in thickness, is partitioned off into a number of **cells** of polygonal form, which are in close connection with one another, so that no spaces intervene between them. Note the green granules (**chlorophyll-corpuscles** or **chloroplasts**), which are here to be seen in considerable numbers in each cell.

Examine the preparation under a high power, and distinguish—

1. The **cell-walls**, which are thin, highly refractive, and of almost uniform width throughout: the extreme margin of the prothallus will be found best adapted for this observation.

2. A colourless film of granular **protoplasm** (primordial utricle), which is in close apposition to the cell-wall, and surrounds a large central cavity (the **vacuole**) full of colourless cell-sap: in this protoplasmic film are embedded—

3. The **chlorophyll-corpuscles** or **chloroplasts,** which will now be seen to be flattened disk-like bodies.

4. A single, more highly refractive **nucleus** is to be found in each cell, its position being variable.

Treat the preparation with *iodine* solution, and observe that—

1. The protoplasm will be stained brown.

2. The chlorophyll-corpuscles, for the most part, a dusky purple.

3. The **nucleus** will be more deeply stained, and will

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accordingly be more easily recognized. One or more roundish, highly refractive bodies may be seen in the nucleus (**nucleoli**).

4. The cell-walls are not stained.

a. Treat a fresh preparation with potash solution, and warm gently over a spirit-lamp: observe that the protoplasm, chlorophyll-grains, and nucleus lose their definite outlines, and, undergoing a process of swelling, become at the same time more transparent. This may best be seen in a specimen which has been bleached in alcohol.

b. Mount another preparation in "eau de javelle" (see Appendix A), and observe it at intervals for some minutes: a similar result will be seen, viz. the contents of the cells swell, and the whole tissue becomes more transparent: this is especially the case in the region near the apex of the prothallus.

c. Irrigate a fresh specimen with a $2\frac{1}{2}$ per cent. solution of common salt, and watch the result: it will be seen that the protoplasm contracts, often taking the form of an almost spherical ball, thus separating from the cell-walls with which it was originally in contact: the latter will now appear as a continuous network of partitions dividing the whole prothallus into a number of chambers.

If this preparation be examined under a high power, a number of delicate protoplasmic filaments may be seen connecting the outer surface of the contracted protoplasm with the cell-wall: this indicates that the two bodies are not merely in apposition in the living cell, but are closely connected.

A cell in this state is said to be **plasmolytic**: the

contraction is due to the withdrawal of water from the cell-sap by the salt solution, this withdrawal not being compensated for by the entrance of salt solution into the vacuole. The salt solution diffuses through the cell-wall, and occupies the space between the cell-wall and the contracted primordial utricle, but it cannot pass through the primordial utricle to any considerable extent.

On washing the section with water, the plasmolytic cells gradually reassume their normal appearance.

From such observations as these it is concluded that the passage of substances in solution into or out of the protoplasm is controlled by the primordial utricle so long as the cell is living.

IV. These **osmotic properties** of the cell can be easily studied in cells which have coloured cell-sap, such as those of the garden Beet: this will at the same time serve as a first exercise in cutting sections from a solid mass of tissue.

Cut a transverse section (p. 7), of a piece of a fresh Beet-root sufficiently thin to be transparent, but of such thickness that at least some of the cells shall remain uninjured, and mount in water: observe under a low power—

1. The thin **cell-walls.**

2. The layer of **protoplasm** (primordial utricle), which lines the cell-wall.

3. The red **cell-sap** filling the cavity of the cell (**vacuole**). Note that the red sap does not escape from uninjured cells.

a. Examine a similar section which has been dipped for a moment into alcohol and thus killed: the red sap

diffuses out of the cells: hence it is evident that though the colouring-matter does not diffuse out of a living cell, it diffuses readily out of a dead cell.

b. Mount another section in water, and run some 2½ per cent. salt solution under the cover-slip; it will be seen that the red sap collects as rounded deeply-coloured bodies in the centre of the cells. This is due, as in the previous cases, to the contraction of the primordial utricle. Wash out the salt solution with water, and some at least of the plasmolyzed cells will gradually reassume their original appearance.

V. In order to observe the **movements of protoplasm** in the living cell, mount in water a rootlet of *Trianea Bogotensis*, or, if that be not available, of *Hydrocharis morsus-ranæ:* examine it first with a low power, and note the solid cylindrical body of the root with its conical apex: its surface is studded, except near to the apex, by transparent cylindrical out-growths —the **root-hairs.** Neglecting the body of the root, focus under the high power upon one of these hairs, and observe:—

1. The thin, smooth **cell-wall.**

2. The granular protoplasm constituting the **primordial utricle** which lines it, and surrounds the large central **vacuole,** from which it is separated by an irregular inner surface.

Focussing carefully upon the granules in the protoplasm, these may be seen to be in motion, being carried along by a streaming movement of the protoplasm (**rotation**). By gently warming the slide on the palm of the hand, or over a lamp, the movement may be accelerated.

Heat the slide over a spirit-lamp to boiling-point: the movements will, on examination, be seen to have stopped, the cell having been killed by the high temperature.

Treat another preparation, in which active movement is going on, with iodine solution: the movement will be arrested, the cell being killed: the protoplasm will be stained brown.

Similar movements of rotation are to be seen more or less clearly in living cells generally, and are easily observed in cells of the leaf of *Vallisneria spiralis*, *Elodea canadensis*, and especially well in the large internodal cells of *Nitella*, &c.

More complicated movements are to be seen in various hairs, and notably in those which cover the base of the stamens in species of *Tradescantia*.

Remove a few of the hairs from a stamen of an open flower, and mount them in water. Observe under a low power the moniliform hairs, each composed of a row of barrel-shaped cells. Focus the high power upon one of these cells, and note the limiting **cell-wall,** and **protoplasmic lining:** threads or **bridles** of protoplasm, irregularly disposed, pass from the peripheral protoplasm towards the centrally disposed, spherical **nucleus.**

Examination of these threads will disclose movements of the protoplasm in various directions: these more complicated movements are collectively termed **circulation.** The hairs should be treated as above directed in the case of the root-hairs, to show that the movement depends upon the life of the cell.

III

COMMON MICRO-CHEMICAL REACTIONS

A FEW further practical exercises will now be given, involving the use of common methods and reagents, and leading to a fuller knowledge of the appearance and reactions of the parts of the cell, and of some of the bodies commonly contained in it.

I. **Cell-walls.**

A. **Cellulose Walls.**

Take some ordinary unbleached "cotton wool," which consists of unicellular hairs, from the surface of the seed of the cotton plant (*Gossypium*). Moisten first with alcohol, and then soak in water.

a. Mount a small quantity in water, and examine first with a low, and then with a high power: observe—

1. The long, filamentous, unicellular **hairs,** which compose the "cotton-wool," coiled irregularly together.
2. The rather thick, highly-refractive and colourless **cell-wall.**
3. The remains of granular **protoplasm,** which may still be seen within.

b. Soak a small quantity of the cotton for a few

minutes in iodine solution in a watch-glass, mount in iodine solution, and note the cell-walls stained slightly yellow.

c. Mount a small quantity of the cotton which has been thoroughly soaked with iodine in a single small drop of concentrate sulphuric acid diluted with an equal volume of water (the greatest care is to be observed in the use of this reagent, so that it shall not gain access to the stage, or the objective: only a very small quantity is to be used, and *the slide should be washed in water directly the observation has been made*). A low power will suffice to show that—

1. The cell-walls swell greatly, and in an irregular form, and ultimately lose their sharp contour.
2. They assume a blue colour. This colouring is often not uniform, and this reaction, though trustworthy as positive evidence of the presence of cellulose where the blue colour is obtained, is not secure as proving the absence of cellulose if the blue colour is not seen.

Cell-walls, and certain of the cell-contents (protoplasm, starch-grains, aleurone-grains, crystalloids) usually contain in their substance a certain amount of water termed the **water of imbibition**. The amount of this water of imbibition may be made to vary by appropriate reagents, and this involves variation in size of the body observed: if the quantity of water of imbibition be increased (for instance by the action of sulphuric acid as above directed) the body swells, losing at the same time its high refractive power, as in the case of the cellulose wall when treated as above with dilute sulphuric acid. Similar phenomena will be described below in starch-grains, &c. Other reagents may withdraw water, in which case a diminution of bulk will be the result.

d. Mount a fresh piece of the soaked cotton in chlor-

zinc-iodine, and observe that the cell-wall stains a more or less distinct blue or a pinkish violet according to circumstances: the protoplasm, of which a small quantity may remain in the hairs, stains yellow: compare the results of similar staining in the last chapter (p. 27).

In some cases the cell-wall turns blue when it is treated with iodine alone; instances of this are to be found in the cell-walls of the asci of Lichens, the bast in the stem of *Lycopodium* and in the root of *Ruscus*, the endosperm of *Pæonia*, and the cotyledons of various Leguminous seeds.

In other cases the characteristic reactions are not given on treatment with chlor-zinc-iodine, or with iodine and sulphuric acid; instances of this occur in the tissues of young seedlings, of growing-points, of the cambium, and of Fungi. In the case of young tissues it suffices to treat them previously with hydrochloric acid, or with solution of potash for a short time: they then give the reactions mentioned above; the tissues of Fungi require a long treatment (three or four weeks) with potash.

e. Mount still another small quantity of the soaked cotton in acid solution of aniline sulphate, and observe that the cell-walls do not stain.

f. One of the most characteristic reactions of cellulose may be observed as follows:—

Prepare an ammoniacal solution of cupric hydrate (see Appendix A): take, in a pair of forceps, a small quantity of cotton-wool, and immerse it in the fluid: it will be seen that the separate hairs of the cotton lose their identity, coalesce into a gelatinous mass, and are finally dissolved.

The solution, and antecedent swelling of the walls may be observed on a slide under the microscope if a very small quantity of the cotton-wool be mounted in the solution.

The above reactions of cellulose may with advantage be repeated in sections cut from the endosperm of the common Date. Take the "stone" of a dried Date, and clearing away with a scalpel the brown superficial coat, so as to lay bare the pearly endosperm, cut thin sections in a tangential plane, *i.e.* parallel to the external surface. *Very small* sections will suffice, and great care should be taken to cut the sections *thin*, otherwise the razor will be damaged, and the sections will not answer their purpose. It would be well to use only a small part of the edge of the razor for this work, *e.g.* the part nearest the heel of the blade.

Examination first under a low, and then under a high power, will show the mass of homogeneous tissue of the endosperm as consisting of thick, highly refractive cell-walls forming a network with circular meshes: here and there the cell-walls show thin spots—the pits. The circular cavities are occupied by granular protoplasm. The thinnest parts of the sections will show the reactions the best, and the superficial layers of the thick cell-walls better than the middle lamella.

B. **Lignified Walls.**

For the reactions of lignified walls the wood of the Pine will serve: for instance, sections may be cut from an ordinary wooden match. Having cut thin transverse sections, soak them first in alcohol to remove bubbles of air: mount one of them in glycerine, and observe under a high power the very regular network of **cell-walls,** which are of almost uniform thickness, and are colourless or slightly yellow: protoplasm is practically absent in this tissue.

a. Treat a fresh section with iodine solution, and note that the walls stain distinctly yellow.

b. Mount a section thus thoroughly stained with iodine in a single drop of sulphuric acid: no blue colour is produced, the walls swell as do the cellulose walls, but their colour is brownish.

c. Mount a fresh section in chlor-zinc-iodine; the walls stain yellow, with no trace of blue.

d. Mount another section in acid solution of aniline sulphate: the lignified walls stain yellow.

By means of the above reactions a lignified wall may be distinguished from a cellulose wall.

C. **Corky Walls.**

Cut thin sections from a piece of common bottle cork: soak them first in alcohol, in order to remove air bubbles, and then in water: mount a thin section in water, or dilute glycerine, and note under a low power the regular arrangement of the tissue, and the thin, pale yellowish or brown cell-walls, with sharp definition and the absence of cell-contents.

a. Treat a section with iodine solution: the walls stain yellow.

b. Treat another section with chlor-zinc-iodine: the walls stain yellow or brown.

c. Treat, as above directed (p. 36), with iodine and sulphuric acid: the walls are yellow or brown, and do not swell, but retain their sharp outline.

d. Treat a fresh section with Schulze's macerating fluid (see Appendix A), and warm gently at first: the corky walls turn yellow: then boil vigorously (this should be done at some distance from the microscope as the fumes given off are apt to attack the metal),

and on cooling re-examine: the corky walls, if the reaction be complete, will be found to have lost their definite outline, and to have run together into irregular **viscid drops** of ceric acid, which is in some measure soluble in the mixture when hot, and is reprecipitated on cooling.

This reaction may with advantage be performed in the bulk, by cutting some shavings of cork, and boiling them for some minutes in Schulze's macerating fluid: they will be seen to lose shape, and coalesce into a viscid mass: this is soluble in warm alcohol, benzol, &c.

Stratification and Striation of thickened Cell-walls.

If the thickened cell-walls of the endosperm of the Date, or of the wood of *Pinus*, or of the superficial layer of the testa of *Linum*, be examined under a high power, they will all be seen to show more or less clearly a **stratified structure**, being composed of successive concentric layers: this may be more clearly seen in various other specimens, a good one being a transverse section of an old branch of *Clematis Vitalba;* mount in water, and examine with a high power.

Observe the thick-walled cells of the pith; the wall appears to consist of a series of concentric layers; this appearance is described as the **stratification** of the cell-wall.

Strip a piece of the bark from the branch, and remove with a needle some of the fibres which compose the internal layer of the bark; mount in water, tease out with needles, and examine with a high power.

Observe the dark lines running in the wall of the fibres at an acute angle to the longer axis of them; note that these lines run in different directions in different layers of the wall of the fibres; this may be seen by carefully focussing first the superficial, and then the deeper layers of the wall; these lines are described as constituting the **striation** of the cell-wall.

The lines of striation may be very well seen in longitudinal sections of the wood of the Pine.

Double Refraction of Cell-walls.

In order to study this subject, apparatus for polarizing light must be adapted to the microscope. This consists of two Nicol's prisms, one of which is fitted into an eye-piece, the other being fixed below the stage of the microscope, so that the light which is reflected from the mirror must pass through it: the former prism is termed the analyzer, the latter the polarizer.

The sections to be examined may be mounted in water or in glycerine, but the best results are obtained with sections mounted in Canada balsam. A twig of almost any tree affords good material for observation. A thin, nearly median, longitudinal section is to be made and mounted: a high power must be used.

The examination is to be commenced by rotating the analyzer, so that the field of the microscope is bright: the section will then appear much as it does when examined with an ordinary microscope.

The analyzer is now to be rotated until the field is quite dark: it is then seen that the outlines of the cells appear bright, the thick, dense cell-walls (those of the fibres and vessels, for instance) being brighter than the thin cell-walls (those of parenchymatous cells).

This observation indicates that the cell-walls, but not the protoplasmic cell-contents or the cell-sap, are **doubly refractive**, and that the denser the cell-wall the more highly refractive it is.

A thin transverse section examined in the same way is seen to present similar appearances.

It will be observed, in addition, that the transverse section of a much thickened cell-wall (that of a bast-fibre, for instance) presents, when the field is dark, a dark cross: when the analyzer is rotated through an angle of 90°, the dark cross is replaced by a bright one, the field being also bright.

It will also be seen that in examining sections in polarized light thick stratified cell-walls (particularly sclerenchymatous cells) are coloured: this is most apparent when the field is dark. This coloration is due to **interference** of light.

The phenomena of interference can be best studied by introducing a plate of selenite between the polarizer and the analyzer: it is to be placed on the stage of the microscope beneath the

object. Various kinds of selenite-plates may be used: it is assumed here that the plate shows red and green tints. Mount a section of a twig or of a leaf-stalk; rotate the analyzer so that the field is red or green. The interference colours will not be well seen in the thin cell-walls; they will appear merely red or green. The thickened cell-walls will exhibit a play of colours which differs in different cases.

Mount a section of part of a succulent leaf (*Aloe*, *Crassula*, *Sedum*, &c.). Observe that the interference colours in the cuticularized external layer of the outer walls of the epidermal cells are complementary in position to those of the subjacent cellulose layers; this indicates differences of tension in the cuticularized and uncuticularized layers.

II. **Protoplasm and Nucleus.**

The protoplasm of the cell, and the nucleus, may be observed in the living condition as described in the preceding chapter; but in order to recognize the more minute details, and in order to make permanent preparations of these bodies, more complicated methods of treatment are necessary.

The protoplasm and nucleus must first be fixed and hardened (see above, p. 4): the best hardening agent is absolute alcohol; if picric acid be used it must be very completely washed out from the tissues before staining.

Harden the young flowering stem of a common Hyacinth, not more than three or four inches in length, in alcohol: cut longitudinal sections of the basal portion of it, and stain with Kleinenberg's hæmatoxylin (see Appendix A) till the sections are deeply coloured, then wash thoroughly with absolute alcohol in a watch-glass: transfer them (drying off all superfluous alcohol with blotting paper) to oil of cloves, or turpentine and creo-

sote (see Appendix A), in which they should be left for some minutes, so that the fluid may thoroughly permeate them: then mount in Canada balsam dissolved in benzol.

Examine sections thus treated under a high power, and observe the chief bulk of the tissue to consist of square or oblong cells of considerable size: the following parts are to be recognized:—

1. The **cell-wall,** which is uniformly thin and is stained.

2. The protoplasmic lining or **primordial utricle,** which is also stained.

3. A large central **vacuole,** which is not stained, and is usually traversed by fine bridles of slightly stained granular protoplasm: these suspend in a central position—

4. The **deeply stained nucleus:** it may be observed in many cases that the nucleus does not occupy a central position, but is embedded in the peripheral protoplasm, while the whole cell-cavity is occupied by a large vacuole.

Examining the nucleus more closely there may be distinguished—

a. Deeply stained fibrillæ, forming apparently a convoluted coil, or a reticulum: this is the **chromatin.**

b. An unstained matrix in which the fibrillæ are embedded—the **achromatin.**

Pith of a very young shoot of the Elder will also serve as good material for these observations; the young shoot should be treated as above directed, and longitudinal sections will afford similar results. As an alternative method of preparation, which has the

advantage of simplicity, stain the sections from material hardened in alcohol, with a solution of methyl green in weak acetic acid, wash with weak acetic acid, and mount in dilute glycerine: the nucleus only is distinctly stained in this case, but the results are as a whole less satisfactory than when the former method is used.

In the sections above described numerous examples of **dividing nuclei** will be found, especially if the material be fixed immediately after being cut, and if the plants had been previously kept at a high temperature. For further details of the course of nuclear and cell division see below.

III. **Starch.**

a. Scrape the freshly-cut surface of a Potato tuber lightly with a knife, and mount a small quantity of the scrapings in water: examine first with a low, and then under a high power, and observe scattered through the water a large number of somewhat ovoid, colourless, bright-looking, *i.e.* highly refractive bodies: these are **starch-grains;** near to one end, which is usually slightly pointed, is a round clear spot, the **hilum.** The grain will show a **stratified** structure: the layers of stratification near the hilum are almost circular and concentric; the more external layers are excentric and elliptical, and are wider on the side further from the hilum; many of them between the hilum and the broader end of the grain are incomplete; hence the layers are more numerous between the hilum and the broad end than between the hilum and the pointed end of the grain.

Here and there may be seen a **compound grain,** consisting of two small grains in contact by their

broad ends, and invested by several layers common to both.

b. Sections should also be cut from the Potato so as to show the starch-grains *in situ* in the cells. The razor should be wetted with water, and one section (the thinnest cut) should be mounted in water: a section which runs out to a thin edge will be found to be best: examine under a high power, and observe—

1. The numerous **starch-grains** as before.
2. The thin **cell-walls** partitioning off the cells which are of considerable size, and each of them may contain a large number of starch-grains.
3. The **protoplasm,** which is so scanty as often to escape observation.

c. Mount a small quantity of starch-grains in water as before, and irrigate with iodine solution: the starch granules will stain a more or less deep blue according to the strength of the solution: this is the characteristic reaction of starch.

d. Treat another preparation of starch with strong chlor-zinc-iodine: the starch-grains will as before assume a blue colour, but they also swell, and lose their bright, high refractive properties. This fact is to be borne in mind when treating tissues containing starch with this reagent.

e. Mount a fresh slide of starch in water, and irrigate with solution of potash: observe that as the reagent gains access to the granules they swell, and at the same time assume a dull appearance, their high refractive power being lost as they take up additional water of imbibition under the influence of the reagent.

Now wash out the potash thoroughly with water, and irrigate the preparation with iodine solution: the swollen grains will still stain blue, though much paler than before, showing that the swelling with potash does not fundamentally alter the nature of the starch.

f. Mount some fresh starch in water, and heat it over a spirit-lamp till it boils: on examining under the microscope, the grains will be seen to have swollen and lost their high refractive power, forming the starch-paste such as is used for starching linen: staining with iodine will produce the blue colour, and show that they are only swollen, not dissolved: compare the effect of potash. A temperature of about 65° C. is sufficient to cause this swelling.

g. Treat a small quantity of the fresh grains with a relatively large bulk of chloral hydrate and iodine (see Appendix A): they will be seen to swell slowly and give the blue reaction.

h. Digest starch-grains in saliva for some hours at a temperature of about 45°–55° C. Examine them subsequently under the microscope: they will be found to have lost their high refractive power. Stain with iodine: they give a pale blue or yellowish colour.

i. Treat some starch, which has been kept in strong alcohol for some time, with tincture of iodine: the characteristic blue stain will not be obtained, or only to a slight degree: thus it is to be remembered that the blue reaction of starch with iodine is dependent on the presence of water.

j. Mount some starch-grains in a single drop of strong sulphuric acid, and observe quickly under a high power: note that the grains retain their outline, while a dark-looking star-shaped crack is seen occupying the place of the hilum: this is caused by the withdrawal of water from the grain by the acid, the most

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watery part about the hilum contracts more than the less watery superficial layers, and a crack is the result.

Now add water so as to dilute the acid : the grains will slowly swell, as with potash, the cracks disappearing first, as the water gains access to the grain.

Optical Properties.—Mount some starch-grains (potato) in water ; examine under a microscope fitted with polarizing apparatus, as described above (p. 41). It will be seen that when the field is dark the grain is bright, and presents a well-marked dark cross ; when the field is bright, the dark cross is replaced by a bright cross. The starch-grain is thus shown to have the property of double refraction.

The relation of the interference colours can be more definitely made out in starch-grains than in cell-walls. In order to study this point, mount some starch-grains (potato) in water ; rotate the analyzer so that the field is red. Assuming that the starch-grain under examination is so placed that its long axis is directed away from the observer, it will be seen that there is a red cross on the grain corresponding in position to the dark cross mentioned above, that the two lateral segments of the grain are coloured yellow, and that the anterior and posterior segments are coloured blue.

IV. **Chloroplasts,** or **chlorophyll-corpuscles.**

Mount a fresh Fern prothallus in water, and note as in the last chapter the several parts of the cells which compose it, and especially the green **chlorophyll-corpuscles,** which are usually of discoid form, sharply defined from the surrounding colourless protoplasm.

Observe here and there granules of oval or biscuit-shaped outline: these are stages in the process of **division,** by which means the chlorophyll-corpuscles increase in number. Drawings of a series of such forms should be made so as to illustrate the process of division.

Treat with alcohol; the green colouring substance

(**chlorophyll**) will be seen to be dissolved out of the granules, but they will retain the same definite outline as before.

Similar observations should also be made on other objects, *e.g.* the thin leaf of a Moss (*e.g. Funaria*), of *Vallisneria*, or *Elodea*. For further details as to chlorophyll see below.

The various other bodies, which are found either having definite form (such as aleurone grains), or in solution in the cell-sap (such as Inulin), will be described as opportunity offers, later in the book: a special section at the end of the description of the Angiosperms will be devoted to the study of the nutritive materials stored in seeds and fruits. The reactions by which the bodies commonly found as components of the plant-body may be recognized are stated concisely in Appendix B at the end of the book.

Remarks on Staining, Clearing, and Permanent Mounting.

Staining.—It is often useful to stain sections in order to bring out certain points in their structure, or to distinguish between bodies of nearly the same refractive index and appearance, but of different nature. A very large number of colouring matters have been used for this purpose, many of which are mentioned in Appendix A: a very few of them will suffice for ordinary laboratory work, and *none are ever to be used without a definite purpose.*

Staining is best performed by placing a few drops of the staining fluid in a watch-glass and immersing the sections in it. The exact strength of the fluid, and the time of exposure of the sections to its action varies in each case, and must be ascertained by preliminary trials. As a rule, when differentiated staining is desired, the best results are obtained by using a dilute solution, and by exposing the sections for a long time to its action; after staining and before mounting for observation it is as a rule necessary to wash the sections in order to remove the superfluous staining fluid; when the staining substance is dissolved in alcohol, the sections are to be washed out with alcohol; when dissolved in water they are to be washed with water: in the case of iodine staining this need not be done as these colourings fade rapidly when the staining fluid is removed.

Clearing the Preparations.—If it is not desired to observe the details of structure of the protoplasm or of the nucleus, the best clearing agent for ordinary use is a solution of potash, either in water or alcohol.

The clearing action of potash is due to the swelling of various parts of the cells and their contents, so that they become more transparent; at the same time it dissolves many of the granules in the protoplasm, and saponifies the oil-drops. The swelling caused by the action of the solution in water is often too great, especially when it is desired to see the cell-walls distinctly; this difficulty may be got over by the use of the alcoholic solution.

After treatment with the aqueous solution of potash, the sections should be washed in distilled water, and

after treatment with the alcoholic solution in dilute alcohol; the sections, in both cases, may be mounted in glycerine: or the sections may be treated at once with a mixture of potash solution and glycerine, but in any case the potash must be washed out before mounting as a permanent object.

If treatment with potash solution does not readily make the tissues transparent, the action of the reagent may be accelerated and intensified by warming over a spirit-lamp. If the action be too strong, and the tissues become too transparent, this may be corrected by neutralizing with acetic acid.

Another method of clearing, which is used in the preparation of growing-points, is to treat sections with calcium chloride. The sections are placed on a slide in a drop of water, and are then covered with some dry powdered calcium chloride; the slide is then warmed over the flame of a spirit-lamp until the water has nearly all evaporated; a drop or two of water is now placed on the sections, and they are to be mounted in glycerine.

Another method which has recently come into use and gives good results, especially in clearing growing-points, is by the use of "eau de javelle" (see Appendix A). The object, either fresh, or after hardening in alcohol or picric acid, is mounted under a cover-slip in "eau de javelle" for three or four to ten or fifteen minutes, according to the rapidity of action of the reagent: very gentle warming over a spirit-lamp will quicken the action: it is then to be carefully washed with water, next with dilute acetic acid, and it may finally be mounted in glycerine.

All the above methods involve the *partial or complete disorganization of the protoplasmic body:* the following method of treatment has the advantage of preserving the structure of the protoplasm and of the nucleus, and it is specially applicable to material in which the protoplasm has been fixed by alcohol, or by picric acid and alcohol. The sections (after staining, if that is considered necessary) should be placed for a few minutes in absolute alcohol; they should then be transferred to a watch-glass, containing either a mixture of turpentine and creosote (four parts of the former to one of the latter), or some oil of cloves; sections which have been stained with aniline dyes are best cleared by cedar-wood oil; they should be left in this for a short time, until they appear to be quite transparent, and should then be mounted in a drop of Canada balsam or Dammar.

Oil of cajeput will do instead of oil of cloves, and is cheaper, but not altogether satisfactory.

Permanent Mounting.—It was pointed out in the previous chapter that objects mounted in water cannot easily be kept, while the objects do not appear so transparent in water as in some medium of higher refractive index. The media most commonly used are glycerine, glycerine jelly, Canada balsam, and Dammar.

Glycerine.—This may be used for objects prepared from fresh material, or hardened with alcohol, &c., and is especially suited to objects stained with ammoniacal solution of hæmatoxylin, carmine, and many of the aniline colours: it is also used for objects cleared by potash, calcium chloride, or "eau de javelle." Dilute glycerine should be used for this purpose, consisting of a mixture of pure glycerine with an equal bulk of water.

A mixture of two parts of glycerine, and one part of glacial acetic acid, boiled together, is useful in some cases; *e.g.* for mounting the tissues of seaweeds, which are liable to swelling.

In order to make the preparations mounted in glycerine permanent, the cover-slip should be fixed to the slide by applying a coating of gold size, Brunswick black, or Canada balsam dissolved in benzol, round its edge with a brush. If a circular cover-slip be used, a turn-table will be found to save much time in this process. Care should be taken that no glycerine is on the slide outside the cover-slip; if any is there it should be removed by means of blotting-paper before applying the varnish.

Glycerine Jelly.—Objects which may be mounted in glycerine may equally well be mounted in glycerine jelly, in which case since the jelly sets firmly, it is unnecessary to use any cement or varnish. The sections should be previously soaked for a considerable time (*e.g.* one or two days) in glycerine so as to remove water or alcohol from them. A trace of carbolic acid should be added to the glycerine jelly in order to prevent the growth of Fungi.

Canada Balsam and Dammar.—These are both highly refractive media, and are thus well adapted for lending transparency to objects. These media are specially suited to sections stained with hæmatoxylin. Water must be completely extracted from the objects before mounting, by treatment with absolute alcohol, or strong methylated spirit; they are then to be transferred to oil of cloves, or a mixture of turpentine and creosote, or cedar-wood oil, and finally mounted in Balsam or

Dammar. It is better to varnish the edges of the cover-slip of Dammar preparations, but this is not necessary for those in Canada Balsam.

The stain produced by aniline colours is apt to fade, so that they are not to be recommended for preparations which are to be kept for a long time. The staining of hæmatoxylin also fades, but more slowly. In order to prevent fading, the preparations should be kept in the dark.

Preparations of green parts of plants in glycerine lose their colour. These may be best put up in a drop of a strong solution of potassium acetate, or of aluminium acetate. The cover-slip must be fastened down as above described.

PHANEROGAMÆ

I. ANGIOSPERMS

VEGETATIVE ORGANS.—(A) DICOTYLEDONS

HERBACEOUS TYPE

Observations with the Naked Eye

I. SOME seedlings of the Sunflower should be bedded out, and allowed to grow for about three months: examine a well-grown specimen of that age, as a whole. The main axis or **stem** is stout, herbaceous, and erect: it often develops to a considerable length without branching: it is cylindrical, slightly striated below, while the higher parts of it, where the lateral branches are developed, are polygonal. Its surface is studded by stiff hairs, which are especially obvious on the lower portions of the internodes.

The stem bears laterally numerous **leaves,** which are simple, petiolate,[1] cordate-acuminate, the margin slightly serrate, ciliated, venation palmate-reticulate,

[1] N.B.—The form of the leaves varies, the lower leaves of the plant being cordate, the upper ones lanceolate with winged petiole.

the surface hirsute. The arrangement of the leaves at the lower part of the plant (and including the **cotyledons,** which wither at an early stage) is opposite, or in whorls of three; higher up, this arrangement merges gradually into the alternate.

The stem is terminated by a **bud,** which may consist only of closely aggregated **foliage leaves,** or it may inclose the **reproductive organs,** which are contained in numerous flowers, closely aggregated so as to form a characteristic **inflorescence**—the **capitulum.** Similar buds, in earlier stages of development, may be observed in the axils of the leaves (**axillary buds**).

Wash the roots and examine them. They are fibrous, and branch profusely. The primary (tap) root, and earlier developed lateral roots are thicker than the later developed roots of a higher order, the latter being successively thinner. This is due to the fact that the roots undergo a process of secondary thickening.

* The Mature Stem.

II. Cut the stem of a well-grown plant transversely at its thickest part, and smooth the surface with a razor.

The most prominent object in the section will be the massive, white, spongy **pith** which occupies the centre.

Around this will be seen, arranged more or less regularly in a circle, and near the periphery, a series of more solid-looking masses of tissue; these are the **vascular bundles.**

III. In order to obtain a clear idea of the course of these bundles, and of their connection with those of the leaves, cut off a piece of the stem, so as to include the insertion of a leaf or **node,** and about two or three inches of stem above and below that point. Bisect this longitudinally in a plane perpendicular to the median plane of the leaf. Clear away the pith with some blunt instrument, taking care not to injure the vascular bundles. This process will be made easier if the stem be previously boiled in water for about ten minutes.

Now dissect out carefully the course of the several vascular bundles, clearing away as much of the internal parenchyma as possible.

Treat the whole preparation with acid solution of aniline sulphate for about five or ten minutes. The vascular bundles will be stained **yellow,** and their course may then be more readily followed. As in Dicotyledons generally, there are here no **cauline** but only **common** bundles: this point will be demonstrated by a study of the apex of the stem (see below, pp. 82, 112).

It will be apparent that in the internodes the bundles run **parallel to one another,** and as a rule without lateral fusion. This regularity is disturbed at the nodes (*a*) by **lateral fusions** of some of the bundles, but not of all of them, and (*b*) by the **entry of fresh bundles** from the leaves (usually three from each leaf), into the vascular ring.

IV. In a longer piece of the stem follow carefully the course of several of the bundles entering from the leaves, as far as they can be traced independently and without fusion. This will be possible at least for **one internode,** and usually for two or three; but the dis-

tance through which this independent course can be traced is variable in this plant. Further, the lateral fusions do not occur only at or near the nodes; instances may not unfrequently be found of fusions occurring at various points in the internodes.

That the arrangement and course of the vascular bundles in the dicotyledonous stem are connected with the arrangement of the leaves is an obvious fact. It may be seen in *Helianthus*, but is more prominently shown in plants with regularly decussate leaves (*Cerastium*, *Clematis* (Fig. 3), *Stachys*). Still the arrangement of the bundles may differ radically from that of the leaves, and is to a certain extent independent of them. This may be seen in such a case as that of *Iberis amara*, where the bundles do not run longitudinally, but in tangential spirals which have no direct relation to the arrangement of the leaves (Naegeli) (Fig. 2). The arrangement of the bundles in the normal dicotyledonous stem in a cylinder is due to the fact that each bundle as it enters from the leaf passes towards the centre of the stem for a certain distance only, which is approximately equal for all; each then curves gradually into a longitudinal direction. As regards the bundle-arrangement, *Helianthus* is not a very good type of an herbaceous Dicotyledon, still it illustrates the most essential points, such as (1) the ring of vascular bundles as seen in transverse section; (2) the entry of the bundles of the leaf-trace between the bundles connected with the higher leaves; (3) the lateral fusion of the several bundles at the node. Since the fusions often occur at points other than the nodes, and since the independent course of the bundles of the leaf-trace is of variable length, it cannot be regarded as a perfect type. Other types are therefore recommended for investigation, in which the vascular system has been carefully traced by Naegeli. In most of these it may be seen how closely the arrangement of the bundles is connected with (1) the arrangement of the leaves, and (2) the number of bundles entering the stem from each leaf. *Iberis amara*, leaves alternate, leaf-trace with 1 bundle (Fig. 2). *Lupinus*, leaves alternate, leaf-trace, with 3 bundles. *Cerastium*

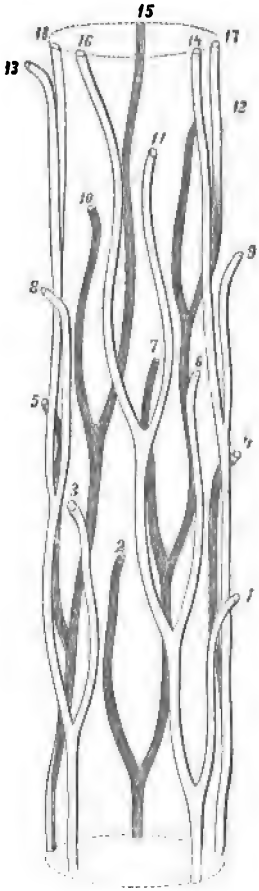

FIG. 2.—Diagram illustrating the course of the vascular bundles in the stem of *Iberis amara*: the points of exit of the solitary vascular bundles of the leaf-traces into the successive leaves are numbered 1—18; the divergence is $\frac{5}{13}$. This figure is constructed from a model prepared in the Botanic Institute in Graz. (After Reinke.)

leaves opposite, leaf-trace with 1 bundle. *Clematis*, leaves opposite, leaf-trace with 3 bundles (Fig. 3). *Stachys*, leaves opposite, leaf-trace with 2 bundles.

The method which has above been recommended in *Helianthus* is a coarse one, and only available in stout herbaceous Dicotyledons. When such a method is used it should always be checked by comparisons of longitudinal sections of the apical bud. As a rule the subject should be studied in the first instance by making such longitudinal sections. These should be thick, and be cleared by treatment with dilute potash, or "eau de javelle." Where the bud is not too bulky, Naegeli adopted the method of bisecting the bud, clearing with potash, and drawing the bundle-arrangement in the two halves; the whole bundle-arrangement at the apex can be deduced from two such sections. As a further control, series of transverse sections should be cut through the apical bud; the order of these and their relative position must be accurately marked. A diligent comparison of these (with drawings) will supply the data for deducing the whole bundle-system. Finally, the results obtained by these two methods should coincide, if the observations be correct.

An examination of the vascular system of a young seedling of the Sunflower will serve as a simple example of the method of study of a bundle-system by means of successive transverse sections.

Take Sunflower seedlings in which the cotyledons have escaped from the seed, but in which the plumule is not yet far advanced: after treatment with alcohol, soak them for twelve hours in dilute glycerine.

Prepare a slide, by covering it, where the cover-slip will be placed, with a thin layer of glycerine jelly: now wet a razor with dilute glycerine, and cut successive transverse sections through the base of the cotyledons and the upper part of the hypocotyledonary stem, keeping them carefully in their right order on the razor: transfer them in that order to the slide, warm gently and cover: compare the sections under a low power, and make drawings of them. There may be slight differences in detail, but the main points to be noticed are these:—

1. The leaf-trace of each cotyledon consists of one median

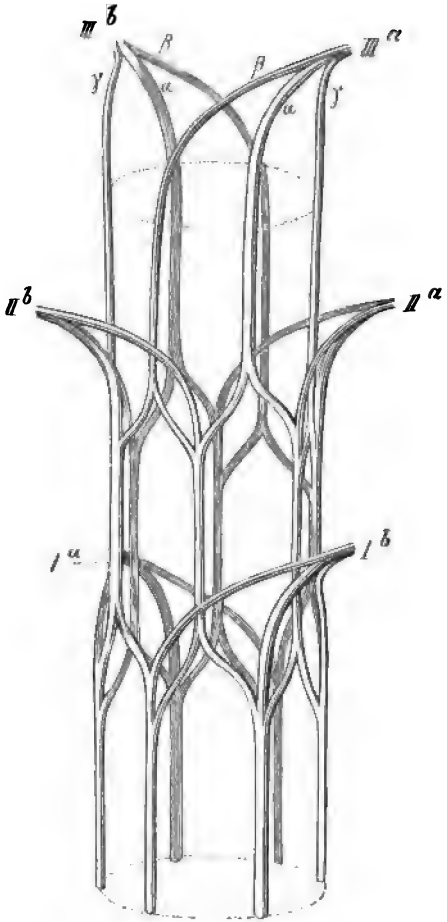

FIG. 3.—Diagram illustrating the course of the vascular bundles in the stem of *Clematis integrifolia*: each leaf-trace of the opposite pairs of leaves consists of three bundles: α is the median bundle, β and γ are the lateral bundles of the uppermost pair of leaf-traces. This figure is constructed from a model prepared in the Botanic Institute in Graz. (After Reinke.)

bundle (more or less distinctly double), and four lateral bundles, two on either side.

2. The lateral pairs coalesce more or less completely, so that on entering the stem the leaf-trace consists of three bundles.

3. The lateral bundles of the two opposite cotyledons coalesce after their entry into the stem, so that the whole trace of the two cotyledons together appears as four bundles in the lower sections of the series.

4. The vascular system from the plumule appears as a ring of bundles of indefinite number.

5. The four bundles of the leaf-trace from the cotyledons insert themselves between the bundles of the plumule, and thus enter the ring.

The sections may be made with a microtome, but this is not at all necessary in such a case as this.

The investigation of a bundle-system as above described is a most useful exercise: by thus comparing a number of sections, putting the results together, and so reconstructing in the mind the whole shoot from which the sections were cut, the student will acquire the habit of regarding a section not as a mere network of cell-walls, but as a slice of tissue which had a certain definite position in the plant from which it was cut. This habit of constantly referring the section to its place in the plant, at the same time as its details are being examined, cannot be acquired too early.

Microscopic Observations.

The material should be kept in spirit for some time to remove resin, and air, and to harden the tissues; but this is not indispensable, and fresh material may be used, though it is not so satisfactory.

I. Cut thin transverse sections of a stem of a well-grown plant of *Helianthus, i.e.* of a stem more than half an inch at least in diameter.

Mount some of these in glycerine or glycerine jelly (these may be kept as permanent specimens), and others in chlor-zinc-iodine. Examine these first with a low power, and observe the following tissues in succession, starting from the exterior:—

1. The **epidermis,** a single peripheral layer of cells, not very well defined from the underlying tissues: it completely covers the surface.

The margin is not perfectly regular, but is here and there extended outwards at the regions surrounding the bases of the large **multicellular hairs,** which may be recognized as being products of the epidermis.

Since these hairs are usually injured in cutting the sections, the width of their bases being greater than the thickness of a fine section, in order to see them well thick sections should be made specially, care being taken that the hairs shall not be previously injured before the sections are cut. They will then be seen to be long **conical hairs** with pointed ends, and divided by transverse septa: their bases are embedded in cells of the epidermis and underlying tissue, which together form at that point a small **emergence,** on the apex of which the hair is borne. Other smaller hairs also occur. Compare the description of the apical bud (p. 82).

2. Beneath this single epidermal layer lies a band of tissue, several layers of cells in width, the walls of which are thickened at the angles where three or more cells meet, the cell-cavity being thus made oval or circular in transverse section; this is the chief characteristic of **collenchyma,** of which this is a good type. Below this lies—

3. A band of thin-walled **parenchyma,** in which are dotted here and there **resin-passages.**

Within these tissues of the **cortex** (a general term including the tissues described under the headings 2 and 3) lie—

4. The **vascular bundles,** which are wedge-shaped, and are arranged in a ring: according to the stage of development of the stem, and the point at which the section is taken, the bundles may be more or less completely joined laterally with one another. In old stems, and at or near the nodes, this lateral fusion is most complete: still, under any circumstances, the originally separate bundles can easily be recognized.

5. Centrally, *i.e.* within the ring of vascular bundles, is the **parenchymatous pith,** consisting of thin-walled cells, which have for the most part lost their activity, having no protoplasmic contents, and are filled with air: hence the whiteness of the fresh pith. In material which has been a long time in spirit, the air may have been removed by the alcohol, but this is usually a slow process.

II. Choose out the thinnest of the sections, and examine it with a higher power, starting as before from the periphery of the stem.

1. The **epidermal layer** will be seen to consist of cells contiguous with one another, without intercellular spaces, excepting occasional stomata: the structure of these will be studied in detail in specimens where they are more numerous. The walls, and especially the external and internal walls, are thick, highly refractive, and show a stratified structure. In chlor-zinc-iodine they are blue (cellulose: see p. 37) with the exception of the outermost layer—the **cuticle:** this is a continuous well defined layer, which stains yellow, and

may thus be easily recognized (see p. 39, reactions of corky walls).

The granular protoplasmic contents of these cells (brown, with chlor-zinc-iodine: compare p. 27) are not plentiful, but form a thin layer lining the somewhat rounded cell-cavity.

Chlorophyll-grains are to be found in these cells: this point is to be noted, since in the stems of many plants chlorophyll is absent from the epidermal cells.

The cells surrounding the bases of the hairs are extended in the direction of the radii of the stem and the whole epidermis is at these points pushed outwards owing to luxuriant growth of the underlying tissue: in fact the hairs are each seated at the apex of an **emergence.** The nature of the hairs themselves will be studied later in connection with the apical bud (see p. 82).

2. In the **collenchyma** the protoplasmic body resembles that of the epidermis: chlorophyll-grains are numerous. The cell-walls also are highly refractive, and stain blue with chlor-zinc-iodine (cellulose); they are specially thickened at the angles, where three or more cells meet; in the thickened mass the **lines or stratification** are well seen. There is no sharp internal limit to the collenchyma, but it merges gradually into—

3. The thin-walled **cortical parenchyma,** which differs from the preceding (*a*) in the thinness of its walls, (*b*) its less copious cell-contents, (*c*) the larger size of the cell-cavity, (*d*) the presence of **intercellular spaces,** which result from the splitting of the cell-

walls at the points where three or more walls meet: in the living state they are filled with air, and even in specimens which have been treated with alcohol, air-bubbles may still be found entangled in them.

Observe carefully the **resin-passages,** which occur in the cortical parenchyma. The resin, being soluble in alcohol, has been removed. To see it in its original condition sections may be cut from the fresh stem, and stained with tincture of alkanet. They are **intercellular spaces,** formed by the splitting of cell-walls. The cavity thus formed is surrounded by small, thin-walled **epithelium,** the cells of which divide both radially and tangentially as regards the passage.

The development of the resin-passages may be observed with great ease and certainty in transverse sections of the stem of Ivy (*Hedera Helix*). Cut transverse sections from a stem in which the development of cork has gone so far that the epidermis is beginning to peel off: mount in glycerine. Scattered through the cortex and pith will be found passages already well developed, and having a structure similar to those in *Helianthus*. If the soft bast, which lies immediately outside the cambium, be examined carefully, resin-passages will be found in various stages of development, starting from a group of four cells, with no intercellular space. In older stages the cell-wall will be found to have split at the angle where the four cells meet, while in older stages again the intercellular space appears larger; meanwhile divisions (radial and tangential, the former more frequent) occur in the epithelial cells.

Treat some sections with some staining reagent, such as carmine or hæmatoxylin, and note that the protoplasm of the cells is stained, but no such staining is seen in the resin-passage: this indicates the absence of protoplasm in the passage itself.

Reactions of Resin.—With a razor wet with water cut transverse sections from a fresh young stem of Ivy: mount in water,

and examine under a low power: observe numerous highly refraction globules scattered over the section, or in the tissue: these will be globules of resin, and they are to be found chiefly in or about the resin-passages.

1. Irrigate with alcohol: the globules will be dissolved.

2. Place another section, in which the highly refractive globules can be seen, on a slide: cut a tangential section from the dry Alkanna root and place it with the outer surface downwards on the section to be tested: irrigate with 50 per cent. alcohol, and cover with a cover-glass for about an hour: on removing the Alkanna section and examining, the globules of resin will be found stained red. Obviously the difficulty in this reaction is that alcohol dissolves both resin and alkannin, but 50 per cent. alcohol has only a very slow action on resin, while it dissolves the alkannin sufficiently for the colouring to take place. The sections might be directly stained with solution of alkannin (see Appendix A), but the results are not so good as by the above method.

Note that in the epidermis, collenchyma, and thin-walled parenchyma of the cortex, there occur, especially in stems growing apace, divisions of the cells in a radial direction. Compare the girth of the stem at the upper with that at the lower part of the plant, or that of a young plant with that of an old one. The conclusion will naturally be drawn that the stem increases in girth as it grows older, and since the outer tissues neither peel off, nor do the individual cells increase greatly in width, longitudinal radial divisions of the cells are the only alternative.

Before leaving the cortical tissue it must be noticed that the **bundle-sheath,** which is the inmost layer of the cortical tissue, and which is easy of observation in the younger stem, may be identified also in these sections, though with difficulty (see below, p. 76).

The layer of thin-walled cells abutting directly on the thick-walled sclerenchyma fibres (yellow with chlor-zinc-iodine, compare p. 38) show in their radial walls the characters of a bundle-sheath, *i.e.* (i.) they are coloured brown with chlor-zinc-iodine; (ii.) they resist the action of sulphuric acid; (iii.) they have the characteristic dark dot (see p. 76). This layer may sometimes be traced as continuous round the ring of bundles, but this is difficult, owing to divisions in the cells of the bundle-sheath, similar to those above noticed in the cortical tissue and epidermis.

Treat some thin sections with sulphuric acid. The bundle-sheath and cuticle resist its action, and since they retain their sharp contour, they are thus brought into prominence. Compare the above reactions of walls of the bundle-sheath with those of corky walls, p. 39.

Within this are—

4. The **vascular bundles.** Select one of the largest of these for more minute examination: it will be found to consist of two well-marked masses of thick-walled tissue, peripheral and central as regards the stem, with a transparent thin-walled portion between them. Further, on examining the latter more carefully, it will be seen that the external part of it has thicker walls, and is less regularly arranged than the central portion, and must accordingly be distinguished from it. We have thus four portions of the bundle which, taking them in succession from the periphery to the centre, are named as follows:—

(i.) **Sclerenchyma.**
(ii.) **Soft Bast** or **Phloem.**
(iii.) **Cambium.**
(iv.) **Xylem.**

i. Examine first the **sclerenchyma.** This appears as a half-moon-shaped mass of tissue consisting of elements with rounded cavity, in which may be recognized the remnants of protoplasmic contents. The walls are thick, and **lignified** (yellow with acidulated aniline sulphate, or with chlor-zinc-iodine, see p. 38). They also show differentiation into layers, of which the most prominent is the bright-looking **middle lamella.** Perpendicular to the internal surface of the walls may be seen **pits.**

ii. The **soft bast,** or **phloem,** consists of elements of very different structure and function: these are:—

a. **Sieve-tubes,** which appear in transverse section as the larger cavities of the soft bast: their walls are rather thin and consist of cellulose (blue, chlor-zinc-iodine). Occasionally these cavities will be found traversed by transverse septa, having a punctate appearance. These stain dark brown with iodine solution: they are transverse **sieve-plates.** (See the description of sieve-tubes in *Cucurbita.*)

b. Abutting directly on the sieve-tubes, and appearing as though they had been cut off from the sieve-tube by a longitudinal wall, may be seen smaller cells: these are the **companion-cells,** but they are not readily distinguished.

c. The remaining elements resemble the sieve-tubes in transverse section except in their smaller size, and absence of sieve-plates: these are **cambiform cells,** or **phloem-parenchyma.**

Passing inwards, the distinction of these several constituents of the soft bast becomes more difficult, while the walls are thinner, and the arrangement of

the elements is more regularly in radial rows, till, in the band of thin-walled tissue which borders immediately on the xylem, these characters become very obvious. This band is—

iii. The **cambium,** or active formative layer. Its constituents are cells arranged in radial rows, with thin cellulose walls (blue, chlor-zinc-iodine), and plentiful protoplasmic contents: the tangential walls are the thinnest, hence we may conclude that the most recent divisions have been in this direction, and have been repeated. Occasionally traces of recent radial division will be found, but this is less common. The form of the individual cells varies from oblong to square, as seen in transverse section: in the former case the longer axis is tangential. Trace the radial series outwards into the phloem, and inwards into the xylem: they may often be followed for a considerable distance with certainty. Note how, in passing from the cambium to the phloem or xylem, the cells divide, and how the form of the individual cells is modified. Hence we may draw conclusions as to the development of the different tissue-elements of the mature xylem and phloem from the originally uniform cells of the cambium. For further details see the Elm (pp. 95, 107) which, being a woody stem, and having more definite secondary increase, is a better type for the study of cambium. (Compare Fig. 9, A, p. 107.)

iv. The **xylem** also consists of elements of various structure: of these the most noticeable are—

a. The **vessels,** easily recognized by their large cavity: they are arranged in radial rows, the individual vessels usually decreasing in size towards the central

limit of the bundle. The walls are thick and lignified (yellow with chlor-zinc-iodine, or with acidulated aniline sulphate, see p. 37); they have no protoplasmic contents their further distinctive characters can only be seen in longitudinal sections. **Thyloses** may be observed (see below, p. 75), especially in more central vessels. The vessels are embedded in a mass of tissue composed of two tissue-forms, which, however, are not readily distinguishable in transverse sections: they are—

b. **Xylem-**, or **wood-fibres,** which appear irregular and polygonal in transverse section, and have thick lignified walls: cell-contents are not prominent, or they may be entirely absent.

c. **Xylem-parenchyma**—cells which retain their protoplasmic contents; their cell-walls are lignified, or of cellulose: the latter is the case with those cells which surround the more central vessels. This constituent of the bundle is more characteristically represented in the stem of the Elm (see below, p. 96).

5. The **pith** consists of cells, which have for the most part lost their cell-contents: they have very thin walls; the walls are slightly pitted: intercellular spaces small. The cell-cavity is usually filled with air, which replaces the protoplasm; this is especially the case near the centre, hence the whiteness of the pith.

III. Cut radial longitudinal sections of an old stem of *Helianthus*, and choosing such as have passed through a vascular bundle (easily recognized with the naked eye), treat them as above.

Bear in mind the observations already made on the transverse sections, and compare those results with the observations about to be made.

To complete the study of the tissues it would be necessary also to cut tangential sections: in the case of tissues in which the radial differ from the tangential walls, such sections must be made, and the comparison drawn between them and the transverse and radial sections. In the present case, however, this is hardly necessary, since the individual components of the several tissues of this stem appear almost uniform in their tangential and radial aspects.

Starting as before from the periphery, note successively the tissues already observed in the transverse sections. It is but rarely possible to see all the tissues satisfactorily represented in a single radial section, therefore the study of the tissues and of their relative positions should be conducted by comparison of a number of sections one with another.

1. The **epidermis,** consisting of oblong cells, whose walls and contents present the appearance already observed in the transverse sections. Note the disturbance of their normal arrangement around the bases of the larger **hairs.**

2. Beneath the epidermis lies the **collenchyma,** consisting of oblong cells with thick longitudinal cellulose walls (blue, chlor-zinc-iodine), and thin transverse ends: the contents are protoplasm, with a **nucleus** and **chlorophyll-grains.** Below each of the larger hairs the collenchyma gives place to short, thin-walled parenchyma, which, together with the epidermis covering it, forms those **emergences** on the summit of which the hair is seated. Within this is—

3. Thin-walled **cortical parenchyma,** the cells of which are shorter, but wider, than those of the collenchyma; there is, however, no sharp limit between

them: observe transitional forms. The cell-contents resemble those of (2), but there is less chlorophyll.

Note the **resin-passages,** the course of which is directly longitudinal; they therefore appear as longitudinal bands of small, oblong, thin-walled cells (**epithelium**).

The **bundle-sheath** may occasionally be recognized as the layer of cells immediately outside the bundle. Very commonly starch-grains may be detected in its cells.

4. The **vascular bundle.** Supposing the section to have been approximately median through the bundle, the following components will be found to be included in it:—

i. **Hard bast, sclerenchyma,** or bast-fibres which appear in longitudinal section as long **prosenchymatous** cells, occasionally divided by more or less oblique septa. Walls thick, lignified (yellow with chlor-zinc-iodine, or with acidulated aniline sulphate), and pitted: remnants of the protoplasmic contents may be found, especially if the stem cut be not very old.

ii. The **soft bast,** or true **phloem,** consisting of tissues with cellulose walls (blue with chlor-zinc-iodine), and abundant protoplasmic contents: its several constituents are—

a. **Sieve-tubes,** long tubes with thin walls and transverse or oblique septa (**sieve-plates**), the structure of which is the chief characteristic of the sieve-tubes; they are readily recognized in sections treated with chlor-zinc-iodine (or iodine solution) by the deep brown coloration of the protoplasm, which is collected round the sieve-plates.

Treat some sections with potash: the protoplasm, and mass of **callus** surrounding the sieve-plates, swells, and the perforated or sieve-like character of the septum, which does not swell, is then easily recognized. Sieve-plates occur occasionally on the lateral walls, where two sieve-tubes are contiguous. The sieve-tubes will be more easily recognized in sections which have been stained with eosin (see Appendix A) which stains the contents of the tubes deeply.

A more detailed study of sieve-tubes, and their structure and contents will be given below in a special section (p. 113).

b. Side by side with the sieve-tubes may be found the **companion-cells,** which are smaller sister-cells of the segments of the sieve-tubes, cut off during development: these are, however, difficult to distinguish, but their presence is proved by the transverse sections.

c. **Bast-parenchyma,** or **cambiform cells.** These are oblong parenchymatous cells with thin, indistinctly pitted, cellulose walls, and protoplasmic contents.

iii. The **cambium,** a narrow band of oblong cells with very thin walls, and dense protoplasmic contents. As the tissue in this case differs in no essential point from that in other plants treated elsewhere, and as it is here difficult to study, its description will be deferred, though its presence here must not be forgotten (see below, p. 103, and Fig. 9, B, p. 107).

iv. The **xylem,** consisting of—

a. **Vessels,** which are its most prominent constituent. They are elements with lignified walls (note reactions as on p. 38), which are variously marked: they have no protoplasmic contents, their wide cavity containing

during life water or gases. The cavity is continuous owing to the partial or complete absorption of the transverse or oblique septa. Note instances of this partial or complete absorption. According to the various markings, or thickenings, of their walls, the vessels may be grouped under the following heads, the first named being the nearest to the periphery of the stem :—

a. **Pitted vessels,** which are the largest, having very wide cavity: their walls are marked with **pits** which appear oval in surface view, and which have the same characters as the round bordered pits of Pinus.

Having observed the pits in surface view, focus so as to obtain a longitudinal optical section of one of the walls; or better, find a place where the preparation is so thin as to show this in real section. Compare this with what was seen in surface view.

β. **Spiral vessels** found in the more central part of the xylem, those most central having the spirals more closely coiled. Note transitional forms (irregularly **reticulated**) between spiral and pitted vessels.

γ. **Annular vessels** found at the central limit of the xylem, the thickening is here in the form of **rings**; in mature stems these vessels are usually more or less disorganized.

b. **Fibrous cells** (wood-fibres), which are long and pointed: it is difficult to follow one individual fibre throughout its whole length, owing to its taking a sinuous course, the fibres being interwoven one with another: their walls are lignified and pitted: the cell-contents are reduced or absent.

c. **Parenchyma,** which is to be found more especially around the vessels near the central limit of the bundle. The phenomenon of **thyloses** is the result of the encroachment of these cells on the cavity of the vessels. The normal individual cells are oblong with square ends, they have cellulose walls (note their reactions), and retain their protoplasmic contents.

The cells termed **thyloses** (Tüllen) are properly included under the term xylem-parenchyma, being derived directly from this tissue in the following way. When fully developed the vessels have lost their protoplasmic contents and their turgescence ; their walls are pitted, at some points being thin at others strongly thickened. If thin-walled tissue, the elements of which are active and turgescent, abut on such a wall, it is obvious that but slight resistance to the internal tension will be offered at the pits, where the wall of the vessel is thin. As a result the wall bulges at these points, and the cells encroach as papillæ upon the cavity of the vessel. Cell-divisions may occur in these papillæ, and the whole process be continued till the cavity of the vessel is completely filled with a cellular tissue.

Look in the longitudinal sections of the old stem of *Helianthus* for instances of such encroachment of cells upon the cavity of the vessel. Good results may be obtained from the old stem, or root, of *Cucurbita*, and from the stems of *Robinia*, or *Vitis*.

5. The central **pith** is composed of parenchymatous cells, with thin walls consisting of cellulose : the walls are slightly pitted : these cells have lost their protoplasmic contents in many cases, and especially near the centre of the stem. Occasional **resin-passages** may be found in the pith.

* * Young Stem.

IV. Cut transverse sections of a young branch of the Sunflower about one-eighth of an inch in diameter.

Similar sections may be cut from the hypocotyledonary stem, in which case they will be found to correspond in all important points to the following description, but they will differ in some of the details ; thus hairs will be absent, the bundle-sheath will be more obvious, &c. : it will therefore be best to cut the sections, as directed, from a young branch.

Mount in glycerine, and passing from the periphery inwards observe successively under a low power—

1. The **epidermis,** as before a single layer, with hairs of various complexity and shape. Beneath this—

2. **Cortical tissue**, which is more or less clearly differentiated into—

a. **Collenchyma.**

β. **Cortical parenchyma.**

γ. **Resin-passages.**

δ. **Bundle-sheath.**

These severally hold the same position, and have the same characters, as were above observed in the older stem, but the tissues are less bulky, and less clearly differentiated from one another.

The **bundle-sheath** in the young stem is more easily recognized than in the older stem though it will subsequently be still better seen in roots. It is a continuous layer of cells, which have a characteristic **dark dot** on each radial wall : this is due to reflection of light from the peculiar sinuous waves of the

central part of the radial walls. The oblique part of each wave acts as a reflector, so that the greater part of the light is diverted before it reaches the eye: hence the origin of the dark dot. The bundle-sheath lies immediately outside the vascular bundles, curving slightly towards the centre of the stem in the spaces between the bundles. It is more prominent in the hypocotyledonary stem, and especially when this is young. The cells are then filled with starch, and the layer may be readily recognized in sections treated with iodine. Under ordinary circumstances it is brought into greater prominence by treatment of the sections with potash, or "eau de javelle."

Within the bundle-sheath, and arranged in a ring, lie—

3. The **vascular bundles,** which are of variable number, they are wedge-shaped and of unequal size, and are composed of elements essentially similar to those described above in the older stem; but the bundles are here much smaller, they consist of fewer elements, and the tissues are immature: thus the sclerenchyma which lies directly within the bundle-sheath has its walls as yet thin; it may already be distinguished from the soft bast by the more uniform character of its elements: the cambium may be distinguished by the regularity of arrangement of its cells in radial rows. Of the wood only the vessels nearest to the pith will be matured, and between them and the cambium various young vessels may be seen, having large cavity, but the walls not yet thickened or lignified.

Note that, if the stem be young enough, the bundles are not joined laterally as in the older stem, but are separated from one another by broad bands of ground tissue. In slightly older stems the cells of this tissue may be found actively dividing, by tangential and occa-

sionally by radial walls. An **interfascicular cambium** is thus formed, and by the tissues derived from it the vascular ring, as seen in the older stem, is completed. Centrally lies—

4. The **pith,** consisting of thin-walled cells, with sparing cell-contents: thus these cells have not yet lost their activity: compare the older stem, where the protoplasmic contents are replaced by air.

Note on Interfascicular Cambium.—It has been seen that in the Sunflower the bundles are quite separate

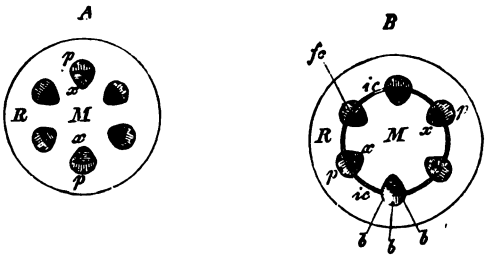

FIG. 4.—A, B. Diagrams illustrating the formation of interfascicular cambium. A shows the primary vascular bundles isolated, and embedded in quiescent ground tissue, before the interfascicular cambium begins to be formed; B shows the interfascicular cambium (*ic*) forming with the fascicular cambium (*fc*) a continuous ring: *p* = phloem; *x* = xylem; *b*, *b*, groups of bast-fibres at the periphery of the phloem. (After Sachs.)

in the young stem, being isolated by masses of quiescent ground tissue. Later, the cells of the latter tissue begin to divide actively as an **interfascicular cambium layer,** lying between the originally separate bundles. This interfascicular cambium joins the margins of the fascicular cambium, and a complete **cambial cylinder** is thus formed. But here in the Sunflower, as in most herbaceous annual plants, the interfascicular cambium is not very long active, the product of its activity being

but a narrow band of secondary fascicular tissue : the identity of the original bundles can thus be recognized at a glance. These points can be very well observed in the stem of *Ricinus* and other herbaceous or semi-herbaceous Dicotyledons. (Compare Fig. 4, A, B.)

The young stem of *Clematis Vitalba* is also very good material for the demonstration of the origin and position of interfascicular cambium.

In some stems (species of *Ranunculus*, &c.) the interfascicular cambium is entirely absent.

In woody perennials (*e.g.* Elm, Pine) the cambial activity begins at a very early period, so that it is difficult at times to recognize the original bundles from the products of the interfascicular cambium, or intermediate bundles : in these plants the activity of the cambium is continued at intervals throughout life. See below, p. 88, &c.

Apical Bud.

V. Take the apical bud of a young plant, or of a young lateral branch of the Sunflower, and cut longitudinal median sections : treat with potash, and mount in glycerine : a better method is to treat with "eau de javelle," and mount as directed on p. 50 : examine with a low power, and then observe—

1. That the axis ends in a naked, broadly-conical **apex** (*punctum vegetationis*), which is surrounded and enveloped by—

2. **Leaves :** these may be observed in various stages of development, the youngest being nearest to the apex :

their order of development is thus **acropetal.** The surfaces of the older leaves are covered with—

3. **Hairs,** which are absent from the apical cone and the youngest leaves, the hairs being developed subsequently to the leaves themselves.

Note (with a higher power) that the apical cone itself consists of thin-walled cells with plentiful protoplasm, which are smaller than the cells of the mature tissues

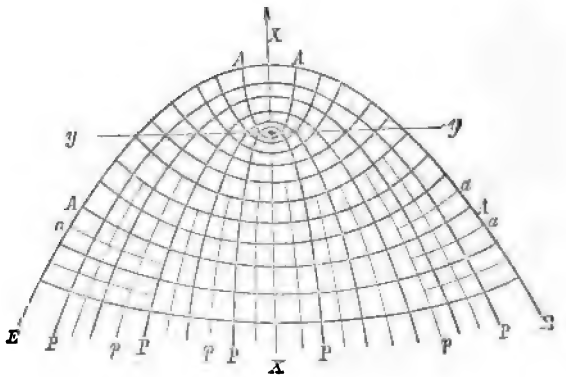

FIG. 5.—Diagram illustrating the plan of arrangement of cell-walls in the apex of the stem of an Angiosperm. X, X = longitudinal axis, which is the organic axis of the stem. E, E = external surface. P,P, P,P, are the periclinal curves. A,A = the anticlinal curves which cut these at right angles. *p*,*p* = incomplete periclinals. *a*, *a* = incomplete anticlinals. The dermatogen is represented by the space between the outer surface, E, E, and the outermost periclinal, P, P. (After Sachs.)

already studied, and are in a state of active division, *i.e.* they are **merismatic.** Observe further that the newly-formed cell-walls cut the pre-existing cell-walls at right angles, and that the two parts of the cells thus divided are apparently equal to one another. A comparison of the general arrangement of the cell-walls with the diagram shown in Fig. 5 will help to make

clear the arrangement of these cell-walls: in drawing the comparison, however, it must not be forgotten that Fig. 5 is a diagram, and cannot be expected to apply in detail.

The whole merismatic mass is differentiated into parts, which may be distinguished more or less clearly from one another, and it will be easy to trace their continuity with the several tissue-systems of the stem and leaves, of which in fact they are the **formative** layers. We may thus distinguish the following:—

1. The **dermatogen,** as a single continuous layer of cells, which divide only in a direction perpendicular to the external surface of the organ (stem or leaf), which it covers completely: it is easily seen to be continuous with the **epidermis,** of which it is the formative layer. Within this is a solid mass of tissue, which looks for the most part dark, owing to its being permeated by intercellular spaces filled with air. It is traversed at a short distance from the external surface by transparent, longitudinal bands of—

2. **Procambium,** which is the formative tissue of the vascular bundles. Trace its continuity with these. Between the procambial bands and the dermatogen lies—

3. The formative tissue of the **cortex** (periblem) which is (partially at least) characterized by dark-looking intercellular spaces.

4. Centrally lies a dark bulky cylinder, which is continuous with, and formative of, the **pith.**

Observe carefully the mode of **origin of the leaves.** They appear at the periphery of the cone as protuberances of the dermatogen and the subjacent cells: the

divisions in the dermatogen are all anticlinal, those in the lower layer are both periclinal and anticlinal. (Compare Fig. 6.) As they increase in size their internal tissues become differentiated into (1) procambium, which is subsequently connected with that of the stem, and (2) tissue with intercellular spaces, which is continuous

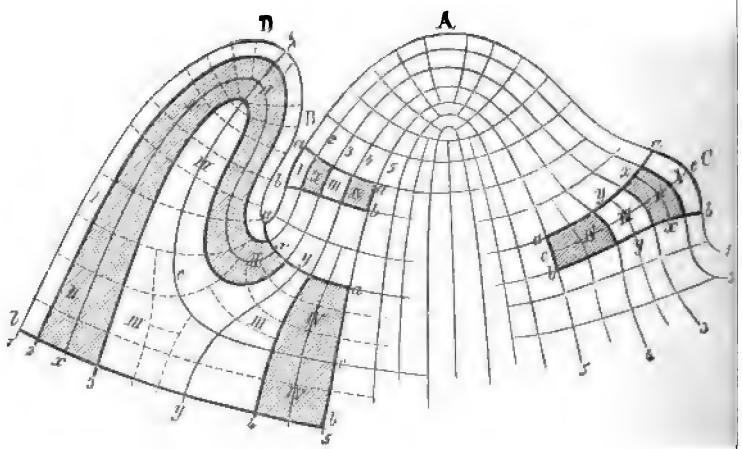

FIG. 6.—Diagram illustrating the mode of origin of leaves (with alternate arrangement) from the growing point of a Phanerogam. A, apex of the growing point. B, C, D, various stages in the origin of a new leaf: the arabic numbers, and shading, indicate how the individual layers of cells take part in the origin of the new leaf. It is clearly indicated in this diagram that no periclinal divisions appear in the cells of the dermatogen. (After Sachs.)

with the cortex. At the same time single cells of the dermatogen grow out, and divide, so as to form the conical multicellular hairs, which cover the surfaces of the leaves. In the older leaves of the bud the development of the emergences around and below the bases of these hairs may be traced: these are not represented in the diagram (Fig. 6).

Note on passing back from the apex towards the more differentiated part of the stem a gradual increase in length of the cells, corresponding to the gradual **extension** of the internodes, while in the first elongated internode of the stem below the bud this is very marked. Observe also the various stages of the process of **vacuolization** of the protoplasm; this will be best seen in sections stained with hæmatoxylin, and mounted in Canada balsam (see p. 52).

Apical buds of the Jerusalem Artichoke (*Helianthus tuberosus*) may be used instead of *H. annuus*, and they have the advantage of being purely vegetative buds. Whereas the Sunflower flowers early, the Artichoke does not flower at all in this country: thus the complications which attend the formation of flowers will be avoided.

Buds of the Common Lilac (*Syringa vulgaris*) afford good material for the study of the apex of the stem: the winter buds may be used either fresh, or hardened in alcohol. The great advantage of this bud is that the leaves are of regular decussate arrangement, and thus by cutting in the median plane of one of the outer pairs of leaves, successive inner pairs will also be traversed in the median plane when the bud is exactly halved. As in the Sunflower, median longitudinal sections are to be cut: treat them for a time (varying with the thickness of the sections) with "eau de javelle"; wash with water, and then with weak acetic acid: mount in glycerine.

In such preparations the apex of the axis will be found to be almost flat, and covered by the continuous dermatogen: the leaves originate from the superficial tissues at its margin, the greatest activity of cell-division being in the hypodermal layer. (Compare Fig. 6.)

In cases where the apical cone is broad, as in *Helianthus*, the tissues, with the exception of the dermatogen, are usually not

sharply defined from one another at a point immediately below the apex ; but the various tissue-systems appear to originate from a common meristem. In some cases, however (especially water plants), the definition is more marked. As an instance may be cited the apex of *Hippuris* (see below, p. 111).

Cell-division.

In the preceding pages reference has repeatedly been made to cell-division, and to meristematic tissues, *i.e.* those in which the cells are actively multiplying by division : observations should now be made of the various stages of the process, and these may be carried out either (1) directly upon living cells, or (2) by examination of tissues which have had their cell-contents suddenly fixed and hardened while the process of division of the cells was in progress.

I. Direct observations of cell-division may be conveniently made by examining the hairs on the stamens of *Tradescantia*. On a warm day take a bud which has not yet attained half the size which it would have immediately before opening : cut away its base transversely so as to remove the receptacle, and press gently between the finger and thumb : the stamens and pistil will be squeezed out : the former are to be placed on a slide in a drop of 2 per cent. sugar solution, remove the anthers and cover the filaments with a cover-glass. Examine under a low power and note the somewhat beaded hairs attached to the filaments of the cells : at, or near to the ends of the hairs, some will be found to contain a single well-defined nucleus, of roundish form ; others of the terminal cells may show a more elongated form, and the nucleus be also elongated and its outline ill defined : the latter are in course of division, and the details of the process may be followed in an individual cell, by watching it under a high power. The hairs thus mounted in sugar solution will continue their normal life for some time, but for prolonged observation it will be better to mount another preparation in a hanging drop of 2 per cent. sugar solution, and keep it in a moist chamber (see Appendix A). The following characters are to be noted in a resting cell as seen under the high power :—

1. The thin cell-wall.

2. The granular protoplasm with one or more vacuoles.

3. The relatively large nucleus, which is sharply defined, and contains fine fibrillæ, which constitute a complicated coiled filament or network of **chromatin** so fine as to give the whole a granular or dotted appearance.

4. One or more highly refractive nucleoli are contained in it.

Note the following changes in a cell undergoing division :—

1. The cell elongates, and the nucleus increases in size : the fibrilla of **chromatin** becomes thickened, and the appearance of the nucleus is more coarsely granular.

2. The fibrilla of chromatin breaks up into a number of short rods which straighten out and arrange themselves in two groups, one on either side of the equatorial plane ; the rods of each group are nearly parallel to one another, and converge slightly towards the poles of the spindle-like figure, which form the whole nucleus has now assumed.

3. The two groups on either side of the equatorial plane separate further, and the rods of each group become connected into the fibrilla of the new nucleus.

4. Fine granules appear in the equatorial plane and constitute the **cell-plate**: they coalesce to form the new septum or cell-wall, which by the widening out of the spindle at the equator, is attached to the outer wall of the cell. The two new cells, each with its nucleus, are thus separated by the new cell-wall which cuts the old wall at right angles.

Other preparations may be made by staining with solution of methyl green in 1 per cent. acetic acid : in these the nuclei will be more distinctly seen : also in those in which the spindle is already formed, fine threads, the **spindle threads** will be seen connecting the two new nuclei, and arranged relatively to one another like the staves of a barrel.

II. Cell-division as it takes place in the multiplication of cells of an ordinary tissue may be observed in the young stems of the Sunflower or Elder. On a warm day, cut young shoots of either of these plants, in which the internodes are between one and two inches in length : fix them at once in alcohol for twenty-four hours or more, and from the tissue thus hardened cut longitudinal

sections so as to traverse the pith. Stain with hæmatoxylin, wash with alcohol, transfer to oil of cloves, and mount in Canada balsam (p. 52). In sections so prepared, if the staining be not too deep, cells may be found whose nuclei may show various stages of formation of the nuclear spindle, the cell-plate, and new cell-wall. The process in its essential points is similar to that above described, and it may be traced by a comparison of the various stages which may be found in the cells of the pith or cortex: pith which is of such age that the cells are not longer than they are broad will give the best results, and care must be taken that the sections be not over stained. Longitudinal sections of the scape of the Hyacinth, not more than three or four inches in length, will also give good results.

Node.

VI. Cut moderately thick longitudinal sections through a **young node** of the Sunflower, so as to include the median plane of the leaf, or of both leaves if they be opposite, as they often are in the lower part of the plant. Treat with potash and glycerine, and warm for a few minutes; or better, treat with "eau de javelle," as directed on p. 50: mount in glycerine, and examine with a low power.

The course of the vascular bundles, which appear dark, is easily followed through the more transparent parenchyma. Note—

1. The continuity of tissues of the stem and petiole; there is no definite boundary between these two parts.

2. That the bundles from the petiole pass into the stem, and, curving at first inwards, they soon assume a longitudinal course.

3. That no bundle of the upper internode lies in the same vertical plane as the bundle which enters from

the petiole, *i.e.* the bundle from the petiole enters between two successive bundles of the vascular ring.

4. If axillary buds be present, note how their bundle-system is inserted on the bundles of the main axis, as well as on those entering from the petiole. This point may be made clearer by a rough dissection of the vascular system of an old axillary bud conducted in the same way as the rough dissection of the leaf-trace (see above, p. 56). Observe the large multicellular hairs seated on the apex of small emergences as before seen (p. 62).

STEM—*ARBOREOUS TYPE*

I. Note the following external characters of a twig of Elm (*Ulmus campestris*) of the current year. It is cylindrical, hirsute, green or brown according to age, the latter colour being due to the formation of a superficial layer of **cork.** Small brown excrescences are scattered over its surface; these are **lenticels.** The arrangement of leaves is alternate and bilateral, phyllotaxis $\frac{1}{2}$, branching axillary: at the base of each leaf, note on either side a small scar, where the stipule was inserted: the stipules usually fall away soon after the buds open in spring, but they may sometimes be found persistent for a time on strong shoots.

II. Cut thin transverse sections of a twig of the current year; mount in glycerine, and examine with a low power. Other sections may, for comparison, be treated with chlor-zinc-iodine, others again with acidulated solution of aniline sulphate.

Observe the general arrangement of tissues in concentric layers, which will be found to succeed one another in the following order, starting from the outside:—

1. **Epidermis:** a single layer of small cells: many of them have grown out, as conical **hairs,** perpendicular to the surface.

2. **Cork:** consisting of one or more layers of square

cells: it will be more strongly developed in older twigs, while it is completely absent in very young twigs: its development is studied below, pp. 91–93. Here and there a **lenticel** may have been cut through, in which case it will appear as a lateral extension of the band of cork.

3. **Cortical tissue:** parenchyma with chlorophyll, having cellulose walls, and intercellular spaces; here and there are large transparent areas: these are cells with swollen mucilaginous walls.

4.[1] Thick-walled masses of **sclerenchyma** (hard bast), which form an irregular broken ring: the walls stain brownish-red with chlor-zinc-iodine.

5. **Soft bast:** a transparent tissue with cellulose walls, and plentiful protoplasm.

6. **Cambium:** a misty layer of thin-walled tissue with plentiful protoplasm: cells in radial rows.

7. **Xylem:** a broad band of thick-walled lignified tissue, with crenated inner margin; centrally lies—

8. The **pith** or medulla: round-celled parenchyma, with thin pitted walls: here and there are large, transparent mucilage cells.

The crenated appearance of the inner margin of the xylem is due to the presence of the wedges of **primary xylem,** forming the so-called **medullary sheath:** the wedges are separated from one another laterally by parenchymatous bands, which may be followed outwards in a radial direction through the whole thickness of the vascular ring: these are the **primary medullary rays:** other rays will also be seen following a similar course, but extending only part of the way from the cambium

[1] 4, 5, 6, 7, together form the **vascular ring.**

to the centre and periphery of the vascular ring: these are **secondary medullary rays.**

Compare with the vascular arrangements of *Helianthus*, and with Fig. 8, *A*, *B*, and *C*.

Cut transverse sections through the axis of a bud, or of a young twig, during the process of extension in spring; treat with potash, and mount in glycerine. In these sections the vascular system will be found to be much less developed, but even here the primary bundles will not be found to be as clearly distinct from one another as in the young stem of *Helianthus*. In ligneous Dicotyledons the interfascicular cambium begins to be active at an earlier period than in those which are herbaceous.

Examine the several tissues, above enumerated, in detail with a high power:—

1. **Epidermis:** a single layer of cells, with the outer wall thickened and cuticularized or corky (test with the usual reagents, see p. 39): **stomata** will be found in a normal position in young twigs, in older ones they are found at the apices of the lenticels (see below p. 93). Note the form of the conical **hairs,** the walls of which are silicified.

To obtain proof of the latter fact, treat tangential sections of the surface of the stem with potassium chlorate and nitric acid dry them with blotting-paper and ignite on a cover-slip, or platinum foil; mount the ash in water, and treat with nitric acid. Silicified walls will after this treatment present the same outline as they originally did. In this case complete skeletons of the conical hairs will be found.

Note on Silicified Cell-Walls.

In many plants the cell-walls over extensive or limited areas are liable to silicification, and by a simple method the **silica-skeletons** of such walls may be prepared, and observed under the microscope. The stems or petioles of the Cucumber afford good

material for the study of such silicified walls. Strip off the superficial tissues, together with the stiff hairs which they bear, and after soaking them well in nitric acid, or in Schulze's macerating fluid (see Appendix A), ignite on platinum foil: treat the ash with dilute nitric acid, and it will not be completely dissolved. Mount some of the residue in water, and examine under a low power: it will then be seen that this residue of the ash consists of skeletons showing the exact conformation of the original hairs, together with a variable area of the tissue which surrounded the bases of the hairs: these skeletons consist of silica, with which the cell-walls must have been completely impregnated. Compare the epidermis of *Equisetum*, which will be described later, and is strongly silicified.

2. The **periderm** (when present) lies immediately below the epidermis: its cells are arranged, in radial rows, without intercellular spaces. Select a thin part of the section for special study of these radial rows, and note in each the following succession of tissues, passing from without inwards:—

a. A series of **cork-cells** as above described: walls stained yellowish brown with chlor-zinc-iodine.

b. At least one cell with very small radial diameter, and with protoplasmic contents, and thin cellulose walls: this is the **cork-cambium,** or **phellogen.**

c. Cells with thick cellulose walls, and protoplasmic contents with chlorophyll: no intercellular spaces: this is the **phelloderm,** which is also derived from the cork-cambium.

Treat a thin section with concentrated sulphuric acid: the walls of all the tissues will swell, and gradually lose their sharpness of outline, with the exception of the cuticularized outer wall of the **epidermis,** and the **cork,** both of which resist the action of the acid. A

similar result may be obtained on treatment with strong chromic acid.

By comparing sections of twigs of various ages, starting from such as have just escaped from the bud, the following facts may be established—

i. The cork-cambium appears in the layer of cortical cells immediately below the epidermis.

ii. These cells divide parallel to the external surface of the stem.

iii. The result of successive divisions in this direction

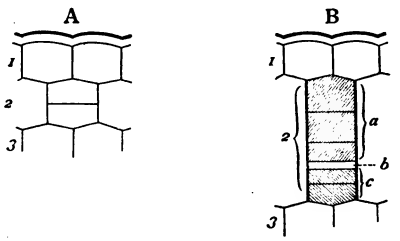

FIG. 7.—A, B. Diagrams illustrating the formation of periderm in the layer of cells (2) directly below the epidermis (1). A shows the first periclinal division of the hypodermal layer (2). B shows as the result of repeated periclinal divisions a radial row of cells, of which the outer portion (*a*) is cork; the inmost portion (*c*) is the phelloderm: these are separated by a single cell (*b*), which represents the cork cambium.

is the formation of secondary tissues, which develop externally as cork, internally as phelloderm.

iv. The true cork-cambium consists of only a single cell in each radial row, from which, by successive division, all these secondary tissues are derived: compare cambium of vascular bundles (see below p. 106, &c.).

v. The cells of the cork-cambium occasionally divide radially.

The diagram (Fig. 7) will help to make this plain

Examine points where a **lenticel** has been cut through, or make median sections through a lenticel.

Note that here the cork layer widens out laterally so as to form a hemispherical mass (semicircular in section), which is covered by the extended epidermis; if the section be median, a **stoma** will usually be seen at the apex of the lenticel: the whole mass of tissue consists of cells of a corky nature, with intercellular spaces.

By comparing sections of twigs of various ages it may be seen that lenticels originate below the stomata, the subjacent cortical tissue dividing by walls both radial and tangential; secondary lenticels are also formed later; these appear at points independent of the stomata.

The lenticels of the Elder (*Sambucus*) are of large size, and are well suited for the study of their typical structure.

3. The **cortical tissue** is a broad band consisting of parenchymatous cells, with intercellular spaces. According to their various characters they may be thus grouped :—

a. Ordinary **parenchyma cells,** with cellulose walls and protoplasmic contents with nucleus, chlorophyll, and starch-granules: the two latter are not constantly present.

b. Cells (idioblasts) with large **crystals.**

c. Large cells whose **mucilaginous walls** almost or entirely obliterate the cell-cavity.

Note that the cells (*a*) are subject to radial division, and that the whole cortical tissue undergoes tangential extension, so as to keep pace with the increasing bulk of the internal tissues.

N.B.—No obvious bundle-sheath is present in this stem.

Note on Mucilaginous Walls.

Soak a few seeds of *Linum* (Linseed) in water for an hour or so. Observe that the surface of the seed, which was originally glossy and hard, is now covered by a pearly layer of transparent, swollen mucilage, of considerable thickness.

a. Cut thin transverse sections of a dry seed (the razor being used dry, or moistened with alcohol, or with pure glycerine): mount in pure glycerine, and examine first under a low power. Neglecting the internal structure, observe the **superficial layer of cells**, of the testa, or outer coat. Note especially their thick, stratified walls, of which a superficial layer (the **cuticle**) may be recognized by its high refractive power, also the **middle lamella**, easily recognized by its optical properties.

Dilute the pure glycerine with water, and observe the thickened walls: their substance will be seen to swell slowly, and the stratified structure to become more apparent. The swelling will often be seen to rupture the outer layer of cuticle, the swollen mass protruding, and constituting the transparent layer seen on soaking the whole seed. Thus the mucilaginous wall, though it swells, is not dissolved. The middle lamella remains as a more or less definite layer, which does not swell.

b. Treat a similar section with potash, and observe the much more quick and complete swelling of the mucilaginous walls. The action is intensified by slightly warming the slide.

c. Treat a section, which has been soaked in water for a few minutes, with iodine solution. The swollen mucilage does not stain to any appreciable extent.

d. Treat a similar section with Hoffmann's blue. The swollen mucilage does not stain appreciably. These preparations will serve to bring out clearly the limits of the swollen mass, which will appear as a transparent zone round the sections, sharply defined from the surrounding coloured fluid.

e. Treat a section with corallin-soda solution: the mucilaginous walls stain pink.

f. Apply the test of iodine and sulphuric acid: a more or less distinct bluish tinge may be seen. This is absent from gums, which are difficult to distinguish from mucilage by microchemical tests.

The cases of the Elm and Linseed are examples of mucilage derived from cell-walls; but mucilage also makes its appearance at times in the protoplasm: as an example may be taken the tuber of Orchis. Cut transverse sections of the tuber of *O. maculata latifolia*, or *mascula*, stain with corallin-soda and mount in glycerine; large cells will be seen in the parenchyma, with mucilaginous contents stained pink, surrounding a central bundle of raphides.

Secretion of mucilage, in the first instance as distinct drops within the protoplasm may be observed in the hairs which cover the young parts of *Blechnum* and *Osmunda*.

4. The **sclerenchyma** consists of cells with walls so thickened that the cell-cavity is often obliterated: the walls are differentiated into two or more strata. Reactions with aniline sulphate, light yellow; with chlor-zinc-iodine, brownish red.

5. The **soft bast** is, as in the Sunflower, composed of several different thin-walled tissue-elements, which are, however, difficult to distinguish in transverse sections: they are—

a. **Sieve-tubes,** which are nearly circular in section, and usually of larger cavity than the other constituents.

b. **Bast-parenchyma:** cells often arranged in more or less regular radial rows: certain of the cells differ from the rest in containing one or more crystals.

The nature of these several tissues will be more successfully studied in longitudinal sections.

6. The **cambium** consists of thin-walled cells arranged, as in the Sunflower, in **radial rows,** which may often be traced outwards into the phloem, and inwards into the xylem: the cells have copious protoplasm, in which an elongated nucleus may often be observed.

Note that the tangential walls are thinner than the radial walls ; also that the radial diameter of the cells is less than the tangential. These facts, together with the arrangement of the cells in radial rows, point to a sequence of divisions, by walls parallel to one another, in a tangential direction. If careful comparisons of a number of different radial series be made, it will be found that the arrangement is such as would result from the action of Sanio's law of cambial division (compare Fig. 9, A, on p. 107).

The structure of the cambium may be very well demonstrated in young stems of *Clematis*.

7. The **xylem** also consists of several different tissue-forms, all of which have **lignified walls** (note their reactions) : they are—

a. **Vessels,** easily recognized by their large cavity, and by the absence of any protoplasmic body. They occur, singly or in groups, scattered through the xylem.

It may be found that the cavity of some of the vessels is filled with a cellular tissue. This is especially frequent in the part of the xylem-ring nearer to the centre : the name **thylose** is given to such cells (see above, p. 75).

b. **Xylem-fibres** or **wood-prosenchyma,** consisting of elements with much smaller cavity, little or no protoplasm, and thick walls.

c. **Xylem-parenchyma,** recognized by the presence of a protoplasmic body, and (at all events in winter) of starch-grains. The cells of this tissue are usually grouped round the vessels, and often form bands connecting two consecutive medullary rays laterally.

The cells of those parts of the **medullary rays** which are in the xylem are thick-walled, lignified, and pitted : they have protoplasmic contents and

starch : they are elongated in a radial direction. Note that they have special cambium-cells, differing in form from the ordinary cambium (compare Fig. 9, A). In the phloem the cells are thin-walled (cellulose), and have plentiful protoplasm.

8. The **pith.** In the peripheral part the cells have thick, lignified, pitted walls, and a protoplasmic body with starch (at least in winter). Tissue of this nature merges gradually into the central tissue with thin walls (lignified and pitted), and no protoplasm. Mucilage cells occur here and there.

There is considerable variety in the structure of the pith of woody plants : the Elm is an example in which the peripheral thick-walled tissue remains active, while the central thin-walled part is inactive : in certain other plants these tissues are mingled together throughout the pith, and this may be very well seen in *Rosa* or *Rubus :* here the thick-walled actively living tissues form a reticulum, the meshes of which are filled with thinner-walled inactive cells.

III. Cut a four-year-old twig of Elm transversely, and smooth the cut surface with a razor.

The age of a twig may be judged externally by counting backwards the annual increments of growth from the apex. The limits of each annual increment of growth may be recognized by the close aggregation of the scars of the leaves or scales at those points.

Examine with a lens, and observe—

1. The **pith,** which occupies the organic centre of the stem. Its position does not, as a rule, coincide with the geometrical centre. Externally to this lies—

2. The **xylem,** which is here a broad yellowish band, clearly marked off into a succession of concentric

rings; these, as a rule, correspond in number to the years of the twig (**annual rings**).

3. The **cambium** lies at the outer limit of the xylem, but it will hardly be recognized as a definite band of tissue under a simple lens, since it is a very narrow zone: its position may frequently be recognized by the rupture of the tissues, the walls of the cambium being thin and easily broken. Outside this is—

4. The **phloem,** which is a much narrower band than the xylem, and is also marked off, though less distinctly, into concentric rings of equal number. Outside this lie—

5. The **cortical tissue** and **cork,** which are usually of insignificant bulk, compared with that of the vascular tissues: in some cases, however, there is an unusually great development of cork, which then appears externally as longitudinal projecting plates.

Note the **medullary rays.** Some of these (**primary rays**) may be traced the whole distance from pith to cortex; others (**secondary rays**) only part of that distance. The latter have been entirely formed by the cambium.

It will be found useful to examine transverse sections of other stems also, *e.g.* that of *Ricinus:* the three- or four-year-old stem of the Lime (*Tilia*) is a remarkably good one for showing the arrangement of tissues after secondary growth in thickness; but the soft wood is not a good type for the study of the more minute details. The general plan of the process of secondary thickening, and the relation of the secondary tissues to the primary arrangement, are made clear by means of the diagrams *A*, *B*, *C* of Fig. 8.

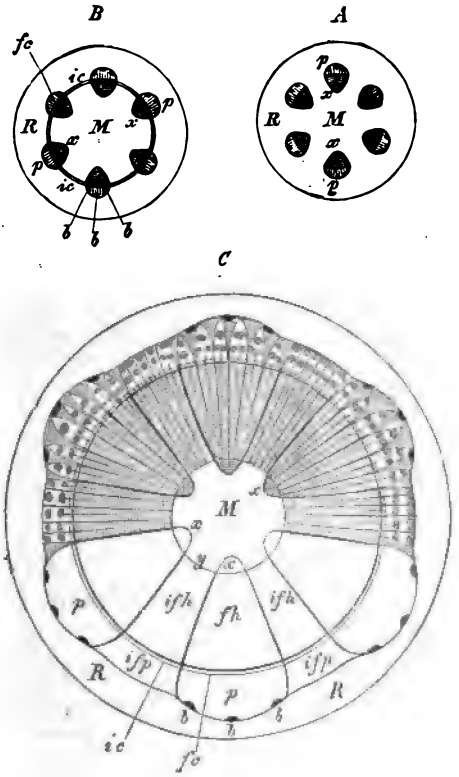

FIG. 8.—Diagrams illustrating secondary growth in thickness in a typical Dicotyledon; the diagrams are based on drawings of transverse sections of the hypocotyl of Ricinus. *A*, *B*, *C* represent the condition of the stem at different stages of development: *A*, before the origin of the interfascicular cambium; *B*, after the interfascicular cambium has been formed; *C*, after the cambium has been active for some time, producing internally a broad ring of secondary xylem, externally a narrower ring of secondary phloem. *R* = primary cortex: *M* = pith; *p* = phloem; *x* = xylem; *b*, *b*, *b* = three groups of bast fibres at the periphery of the phloem; *f c* = fascicular cambium; *i c* = interfascicular cambium; *f h* = wood developed from the fascicular cambium; *i f h* = wood developed from the interfascicular cambium: *i f p* = secondary bast developed from the interfascicular cambium. By the intercalation of the secondary xylem and secondary phloem, the primary groups of bast fibres, *b*, *b*, *b*, are removed a considerable distance from the primary xylem, *x*, *x*, though in the young stem *A* these are in close proximity to one another. Note also in *C* the primary medullary rays, which extend the whole distance from the periphery of the ring to the pith, while the secondary medullary rays only extend through part of that distance. (After Sachs.)

IV. Cut thin sections from the transversely cut surface, so as to include all the bands of tissue from the pith to the cortex: moisten them with alcohol, and mount in water or dilute glycerine. Examine with a low power.

Note that the constituents of the several tissues, produced during the later years, are similar to those already observed in the first year's stem; also that they are arranged, more or less regularly, in radial rows. This arrangement is best seen in the xylem, and it points to the origin of the tissues from the **cambium.**

Observe that the constituents of the autumn-formed xylem are smaller, and have slightly thicker walls than those formed earlier in the year, also that vessels of large cavity are absent from it. Hence arises the appearance of the **annual rings,** which is easily seen in almost any wood of temperate climates, when the transverse section is examined.

In arboreous stems of considerable age a central portion of the woody mass (**duramen,** or heart-wood) becomes differentiated by texture and darker colour from the more superficial and later formed portion (**alburnum,** or sap-wood): this may be seen in old stems of the Elm, but more distinctly in stems of the Laburnum, where the heart-wood is coloured dark brown, while the sap-wood is light yellow. Compare also the black duramen, and light-coloured alburnum of Ebony, and the red duramen of the Logwood, &c., &c.

As stems grow older, layers of cork appear successively further and further from the external surface: not only the cortex but also the outer and older portions of the phloem are thus cut off from physiological connection with the inner tissue: the term **bark** is applied to tissues thus cut off, together with the cork which forms the physiological boundary.

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The formation of bark may be readily traced in the Elm, by cutting transverse sections successively of stems of increasing age: in those in which the external surface of the stem appears smooth the primary formation of cork which originated from the hypodermal layer will probably be the only one present, and in that case a band of cortical tissue will intervene between the cork and the secondary phloem; but transverse sections should also be cut through the superficial tissues of a stem of which the surface is conspicuously fissured: in these one or more layers of corky tissue will be seen traversing the secondary phloem, while tissues of the nature of phloem will be seen included in the brown effete mass of bark which is outside the innermost layer of cork.

Transverse sections should also be made of the stems of *Vitis* of various ages: here the formation of successive bands of cork takes place at a relatively early age, and their number is greater than in most woody stems.

V. Cut radial sections from a four-year-old stem of Elm; soak them for ten minutes or more in alcohol to remove the air-bubbles, and mount in glycerine. Other sections may be mounted in Schulze's solution, and these will perhaps be found the most useful; examine first under a low power.

It will be found difficult to cut good sections so as to include the whole radial surface: it is therefore better not to attempt it, but to study the several structures in a number of successive sections, each extending over only a part of the radial surface.

Starting from the outside, observe the same succession of tissues as already seen in the transverse sections, viz.:—

1. **Epidermis,** which is often dried up and disorganized.

2. **Cork** (including the **cork-cambium** and **phellogen**), with the short cells arranged in radial rows.

3. **Cortical tissue,** with large **mucilage cells.**

4. **Hard bast,** consisting of long fibres.

5. **Soft bast,** thin-walled elements with much protoplasm.

6. **Cambium,** a misty band; cells not easily defined.

7. **Xylem,** with thick lignified walls, the vessels appearing as large tubular cavities.

8. **Pith,** parenchymatous; its appearance as in transverse sections.

Note the **medullary rays,** which appear as narrow bands of parenchyma, following the plane of section.

Examine these several tissues in detail with a high power.

1. The **epidermis,** when still persistent, shows the same characters as are observed in transverse sections.

2. The **cork** is composed of square cells arranged in radial rows, which are continuous through the **cork-cambium** to the **phelloderm,** the latter presenting much the same appearance as in transverse section.

3. The **cortical tissue,** which is parenchymatous throughout, also appears much the same as in transverse section.

4. The **hard bast** consists of long fibres, with thick walls, and very small cell-cavity: they are distributed in irregular groups among—

5. The **soft bast,** which is characterized by thin walls and protoplasmic contents, and is composed of—

a. **Sieve-tubes,** which are best seen in the part of the phloem nearest to the cambium: they resemble in the main, those of *Cucurbita* (p. 113), but are not so wide; the **sieve-plates** are oblique, and face the radial planes. This is the usual arrangement of sieve-plates

in secondary phloem; but their structure is often more complicated, *e.g.* in *Vitis* or *Tilia* (see below, p. 116). The sieve-tubes may easily be recognized in stems cut in autumn by the masses of **callus** which surround the sieve-plates: this stains brown with chlor-zinc-iodine. For the reactions of the callus, see p. 115. Companion cells are not easily seen.

b. **Bast-parenchyma:** oblong cells with cellulose walls: some contain protoplasm and starch, in greater or less quantity according to the season: others contain crystals: note the **medullary rays** as before.

Passing inwards the differentiation of tissues of the phloem is lost in—

6. The **cambium,** which appears here as a narrow band of cells with thin walls, and abundant protoplasmic contents. The form of the cambial cells is difficult to make out, but a careful observation of a good section will lead to the conclusion that the form of the cell as seen in the radial section is oblong and very narrow, with square ends. Compare the diagrammatic figure (p. 107).

7. In the **xylem,** excluding for the present the medullary rays, observe the following elements, all of which have lignified walls—

a. **Vessels** of various orders, which may be grouped as—

(i.) **Spiral vessels** (**protoxylem**) found at the central part of the xylem, next the pith: they are usually more or less disorganized, being often filled with thyloses.

(ii.) **Pitted vessels,** the lateral walls of which are crowded with bordered pits of essentially the same

structure as those in *Pinus*. These vessels are usually of large cavity.

(iii.) Vessels with both **pitted and reticulate** marking, superposed on one another on the same lateral walls: these vessels usually occur in groups, and are of small bore.

Note in all these, but especially in (iii.), points where transverse or oblique septa have been partially or completely absorbed.

b. **Fibrous cells,** which occur in large groups, between the vessels: they are long, and prosenchymatous, and are intertwined, so that it is difficult to follow them through their whole length. They have little or no cell-contents, and their walls are not pitted.

c. **Xylem-parenchyma:** oblong cells with protoplasmic contents, and starch: walls thick, lignified, and pitted: they occur in longitudinal bands: note their close contact on the one hand with medullary rays, on the other with vessels.

Examine the **medullary rays** in the xylem: they are composed of oblong cells, with their longer axes horizontal, arranged like bricks in a wall: in characters they resemble xylem-parenchyma.

8. The **pith** presents in radial section, for the most part, the same characters as those already noted in transverse section.

VI. Treat some small pieces of the wood of the Elm with a small quantity of Schulze's macerating fluid (see Appendix A) in a test tube, and warm gently till the tissues break up, and the several constituents begin to separate: then wash with water, and mount a very small quantity in water or glycerine.

Some at least of the constituents will be found lying separately, or may be detached by slight pressure on the cover-slip: the true form of the **wood-fibres,** as greatly elongated, spindle-shaped cells, will now be seen. Note also **vessels** of the various types above described, and **xylem-parenchyma.**

VII. Cut tangential sections through the **xylem** of a four to five-year-old stem of Elm, treat with solution of iodine, and mount.

Observe first with a low power—

1. The **medullary rays** of lenticular outline, easily recognized as masses of small thick-walled cells of almost circular form, filled with **starch,** which appears dark blue. This is best seen in stems cut in autumn. Examine the medullary rays closely in the thinnest part of the section, under the highest power, and note the small triangular **intercellular spaces,** which take a horizontal course along the medullary rays, and are therefore cut here transversely. In close connection with these—

2. **The xylem-parenchyma,** the cells of which also contain starch, and are thus easily recognized: note that cells of the parenchyma more or less completely surround—

3. The **vessels,** the walls of which are stained yellow, and present those characters already observed in radial sections. The interspaces are filled by—

4. Masses of **xylem-fibres,** which appear as before.

VIII. Cut tangential sections of the **phloem** of a similar stem: treat as before, and observe—

1. The form and arrangement of the **medullary rays** as in the xylem, but the walls of the cells are thinner, and not lignified: intercellular spaces may be

noted here also, similar to those above described as occurring in the medullary rays of the wood. The cells contain copious protoplasm.

2. **Phloem-parenchyma,** the cells of which differ in their cell contents—

a. Some contain crystals.

b. Others have copious protoplasmic contents.

Both forms will be seen to have been derived by division from original elongated cells with pointed ends, since they are arranged in groups of this form. Compare the form of the cambium cell (IX.).

3. **Sieve-tubes** answering to the description given for radial sections. The sieves are oblique, the form of the successive segments oblong. The sieves are callous (see p. 115), and are easily recognized in sections stained with iodine or eosin.

4. **Bast-fibres** as before in radial sections.

IX. Cut tangential sections through the **cambium** of the stem of Elm : treat with dilute potash or "eau de javelle," and mount in glycerine. Examine first with a low power, and note that the general arrangement is similar to that already seen in tangential sections through the mature tissues, also that the form of the cells, in each part of the cambium-zone, is like or similar to the average form of the elements of the mature portion of wood or bast, which borders on it in a radial direction. Thus the cambium is differentiated into—

1. Cambium, of medullary rays, which appears to consist of roundish cells, resembling cells of the mature medullary rays in form.

2. Cambium, from which all the other tissues are derived, the cells of which have a prismatic form.

To gain a clear idea of the process of secondary thickening, the actual form of the cambium-cells and their arrangement must be recognized : as stated above (p. 7), it is necessary, in order to fully realize the form of a cell as a solid body, to cut sections in three directions at right angles to one another : the cambium-cells

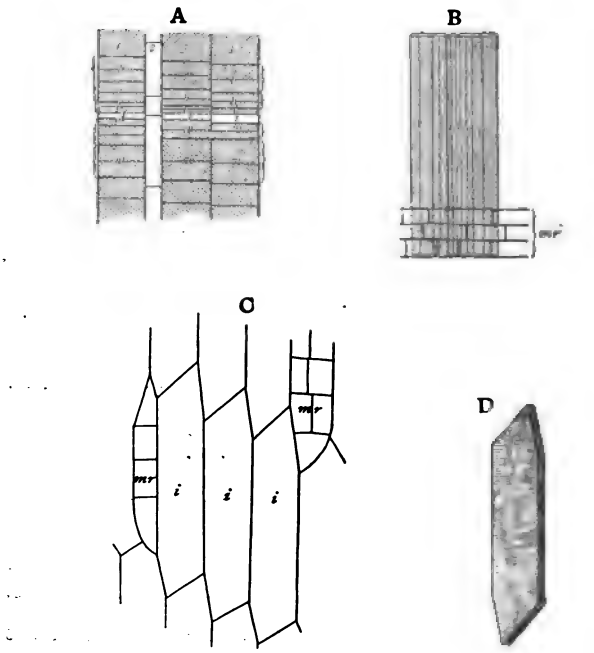

Fig. 9.

have now been seen in transverse, radial, and tangential sections, and the results are represented diagrammatically in Fig. 9, A, B, C, which are based upon results of Sanio's investigations of *Pinus*, but the main points are the same for Dicotyledons.

Fig. 9, A, shows diagrammatically four radial rows of cambium-cells (1, 2, 3, 4,), as seen in transverse section : of these row (2)

is a medullary ray. Note in row (3) the single initial cell (*i*), oblong in transverse section, and the shorter diameter placed radially; according to Sanio's law of cambial division there is only one such initial cell in each radial row: from this successive segments (*w w*) which go to form wood have been cut off on the inner side, others (*b*, *b*, *b*) which go to form bast on the outer side: each is represented in the diagram as dividing into two by a periclinal wall; this is typically the case in *Pinus*, but the division is not so regular in Dicotyledons. In row (1) is represented a segment (*w'*) recently cut off from the initial cell on the side next the wood, in which this division has not yet taken place; in row (4) there is a similar undivided segment (*b'*), which, after division, will go to form bast.

Fig. 9, B, shows diagrammatically the arrangement of the cells of one of these rows (3), as seen in radial section: the length of the cells is much greater than their width, and the ends are square: *i* is, as before, the initial cell of the row: *w w*, pairs of cells formative of wood: *b*, *b*, *b*, pairs of cells formative of bast: *m. r.* is a medullary ray put in so as to show the relative position and form of the cells.

Fig. 9, C, represents the appearance of the cambium cells, *i*, *i*, *i*, in tangential section: they are obliquely pointed, and their width corresponds to that shown in A: *m. r.*, as before, the medullary rays.

Fig. 9, D, shows the form of a single isolated cambium-cell as a solid body, drawn to the same scale as the other figures: if such a cell be cut transversely, radially, or tangentially, it would give the appearance presented by the initial cells (*i*) in Figures A, B, and C.

Taking cells of this form as a starting-point, the several tissues above described are derived from them in the following way:—

(i.) **Phloem.**—*a.* **Sieve-tubes,** by lateral distension, and conversion of the oblique walls into sieve-plates.

b. **Parenchyma,** by division of the cells by transverse septa.

c. **Fibres** (sclerenchyma), by elongation and interweaving of cells, the width of the cells at the same time being relatively reduced: the ends of the cells slide past one another as the cells elongate.

(ii.) **Xylem.**—*a.* **Vessels,** by lateral distension, and absorption of cell-contents, and of the oblique walls.

b. **Parenchyma,** by division of the cells by transverse septa.

c. **Fibres,** by elongation and interweaving of the cells, while the width of the individual cells is relatively reduced.

Observe intermediate stages between cambium-cells and these several mature tissues: this may best be done in sections cut from stems in early summer.

X. To investigate the nature of the **crystals,** several times observed in the parenchyma of the stem of the Elm, cut tangential sections of the **phloem** or of the **cortical tissue,** mount in water, and having found one or more crystals—

(i.) Run some iodine solution under the cover-slip: the crystal is not stained.

(ii.) Acetic acid: it is not attacked.

(iii.) Dilute nitric acid: it is more or less completely dissolved.

These reactions, coupled with what is known from the analysis of ash, point to the conclusion that these are crystals of calcium oxalate.

STEM—*AQUATIC TYPE*

Note the cylindrical smooth stem of the Mares-tail (*Hippuris vulgaris*), bearing whorls of simple leaves. Cut the stem transversely, and note the central vascular cylinder, which is easily seen with the naked eye, and the broad band of cortex with large intercellular lacunæ.

For the microscopic work fresh material may be used: if the material has been kept in alcohol, the sections should, after being cut, be allowed to swell in water before mounting.

I. Cut transverse sections of an internode of the stem of *Hippuris vulgaris:* mount in glycerine and examine with a low power. Observe—

1. A well-marked **epidermis** with cuticle. Here and there are to be seen radiating scale-hairs. These occur especially in the axils of the leaves.

2. **Cortical parenchyma:** a broad band consisting of thin-walled, chlorophyll-containing cells, with large **intercellular spaces.**

3. A well-marked **bundle-sheath,** with the usual characters, which immediately surrounds—

4. The central **vascular cylinder.** This is composed of—

a. A basis of thin-walled **parenchyma,** in which are distributed—

b. In the central part **vessels** of the xylem with lignified walls:

c. Towards the periphery elements with the characters of **soft bast**; the sieve structure is in this case doubtful.

II. Cut thick transverse sections of **nodes**; treat with potash, mount in glycerine; and observe, with a low power, that the distribution of tissues is in the main the same as in the internode, but—

1. The large intercellular spaces are divided by horizontal **diaphragms,** consisting of single layers of cells.

2. Branch-bundles leave the central cylinder, and pass horizontally outwards to the bases of the leaves.

III. Take a terminal bud of *Hippuris:* remove from it the largest external leaves, and then dissect off the inner and smaller leaves with needles in a drop of water on a glass slide: in the centre of the bud will be found the elongated conical and colourless **apical cone.** Cover with a cover-slip, and examine it under a low power: the smooth cylindrical apical cone will be well seen, the inner tissues of it being marked by a reticulum of dark lines: these are the **intercellular spaces** filled with air.

Note especially the **leaves,** which appear as rounded outgrowths laterally on the axis: the larger ones are seated lower down the axis, and they are successively smaller as the apex is approached.

IV. Cut median longitudinal sections of the apical bud of *Hippuris,* so as to pass through the elongated **apical cone;** treat with potash, or with "eau de javelle," and mount in dilute glycerine. Examine first with a low power, and observe—

1. The **axis,** which is wide below, but tapers upwards to the rather elongated **apical cone** (*punctum vegetationis*). The axis is composed of the several tissues already noticed. Note especially in the lower part of the section—

a. The rectangular **intercellular spaces,** divided transversely by **diaphragms** at the nodes.

b. The axile **vascular cylinder,** which may be followed far up into the apical cone, and which gives out lateral branches to the leaves.

2. The **leaves,** diminishing in size towards the apex. Note the **scale-hairs** about the bases of the leaves.

Put on a high power, and examine the apical cone. Note—

1. The **dermatogen,** a continuous layer of cells, which covers the apical cone externally. Trace it backwards from the apex: it will be seen to give rise to the **epidermis.**

2. The **periblem,** consisting of 4–5 layers of cells, which may be traced backwards, and be thus shown to give rise to the **cortex.**

3. A central cylinder of **plerome,** which is continuous with, and gives rise to, the **vascular cylinder** (compare the diagram, Fig. 5, p. 80).

Note that the **leaves** originate from the outgrowth of the dermatogen and periblem, the plerome taking no part in their formation. Also that the vascular system of the stem is already developed at a higher point on the axis than that of any of the leaves. We have thus an instance of **cauline** vascular bundles, that is such as are proper to the stem, as distinguished from **common** vascular bundles, which terminate at their upper extremities in the leaves.

SIEVE-TUBES

i. *Cucurbita*

Though the sieve-tubes of the Sunflower are fairly large, the soft bast does not occur in large masses. In the Vegetable Marrow, however, the sieve-tubes are of extraordinary size, and occur in large numbers: this stem is thus excellently fitted for the study of the sieve-tubes of the type usually found in herbaceous stems.

The material should be hardened in alcohol.

I. Cut transverse sections of the stem of the Vegetable Marrow, stain with eosin, and mount in water or glycerine.

The general arrangement of tissues in this stem differs in several important points from that in the Sunflower, and, indeed, from that in most herbaceous Dicotyledons. Thus it will be seen on examination under a low power that—

1. There occurs at a short distance below the epidermis a thick-walled band of **sclerenchyma** with lignified walls (yellow, with chlor-zinc-iodine, or acidulated aniline sulphate). This is quite distinct from the vascular bundles.

2. The vascular bundles are always separate and distinct: though an interfascicular cambium is formed in old stems, no secondary vascular tissue is derived from it.

3. The structure of the individual bundle is abnormal, there

being in each bundle a central mass of xylem with the phloem masses lying the one on the central, the other on the peripheral side of it: this type of bundle is termed **bi-collateral.** Between the xylem and the peripheral phloem mass is the cambium layer. The structure is fundamentally the same in both phloem masses: either will therefore serve for the study of the sieve-tubes.

In the **soft bast,** which resembles that of *Helianthus* but has larger constituents, observe—

(i.) The transverse, circular, punctate **sieve-plates,** having the same appearance as in *Helianthus,* and easily recognized by their contents being stained with eosin.

(ii.) The **companion-cells,** appearing as though cut off from the side of a sieve-tube by a curved wall.

(iii.) **Cambiform cells.**

Treat some sections with chlor-zinc-iodine: all the walls of the soft bast turn blue (cellulose), but the sieve-plates appear yellow or brown.

II. Cut longitudinal sections through the soft bast: either radial or tangential sections will do. Mount some in iodine solution. The transverse **sieve-plates** will be brought into prominence by the deep yellowish brown staining of the mass of substance, which surrounds them: this may consist of—

a. A **callus-mass,** which surrounds, and often completely invests the sieve-plate: the size of the callus-mass is variable according to season, age, &c., being greatest in autumn, and in old sieve-tubes.

b. **Protoplasm,** which is usually collected in close contact with the sieve-plate (or with the callus if present), and more especially on its upper side.

Note, i. the **oblong form** of the segments composing the sieve-tubes.

ii. The **companion-cells,** short with granular protoplasm, and nucleus.

iii. **Cambiform cells** of similar form to the segments of the sieve-tubes.

Other sections should be stained with eosin, then washed, and mounted in glycerine. The sieve-tubes will be readily seen, as their contents will be stained deeply.

III. (*a*) Mount some sections in water, and having found a sieve-plate with **callus,** run some dilute potash under the cover-slip. The callus-mass swells; the protoplasm also swells: the section thus becomes more transparent, and the **cellulose basis** or true **sieve** becomes more apparent, and its pores can be easily seen.

(*b*) Treat another section in which a callus has been found with Russow's callus-reagent (see Appendix A): the callus stains a deep brown.

(*c*) Treat another preparation with corallin-soda (see Appendix A), and mount in glycerine: the callus stains pink.

(*d*) Stain another preparation with Hoffmann's blue in 50 per cent. alcohol for 24 hours, wash with water, and mount in glycerine: the callus is stained deeply.

IV. Treat some fresh sections with iodine, then dry off the superfluous fluid with blotting-paper, and mount in a single drop of strong sulphuric acid. The cellulose walls and callus will swell; the protoplasm will contract. Look carefully over the protoplasmic contents of the sieve-tubes for the points where sieve-plates have been; here it will be found that fine strings of protoplasm, which passed through the sieve-plate, connect the protoplasmic masses on opposite sides of the

sieve with one another. By this reaction the **continuity of protoplasm** through the sieve is demonstrated.

Another method by which similar results may be obtained is to add a small quantity of dry Hoffmann's blue to a little strong sulphuric acid in a watch-glass, and mix well. Treat the sections with this for a short time, wash with water, and mount in glycerine.

It will be noted that the sieve-tubes of *Cucurbita* closely resemble those of *Helianthus*, the sieve-plates being transverse and simple. This is the usual type of sieve-tube to be found in **primary phloem,** and generally in herbaceous stems of Angiosperms. In the **secondary phloem** of ligneous stems a more complicated type of sieve-tube is frequently found. This will be studied now in the stem of the Lime.

ii. *Tilia* (Lime).

I. Cut radial sections of the phloem of a stem of Lime more than three years old. Stain with eosin, wash, and mount in glycerine; examine them with a high power for **sieve-tubes.** The general arrangement of the phloem is similar to that in the Elm. The sieves, which will appear stained pink, occur on oblique walls facing the radial plane, and are therefore here seen in surface view. Note that they have a similar appearance to those above described, but here three or more sieve-plates occur on each oblique wall.

II. Cut tangential sections of the same; stain, and

mount as before. The oblique walls are here cut longitudinally; the sieve-plates are often **callous,** especially in autumn.

Note the form of the segments of sieve-tubes; it is fundamentally the same as that of the cambium cell as seen in tangential section (compare Fig 9, C, D).

LATICIFEROUS TISSUES

The material for the study of these tissues should be prepared by treatment with alcohol to coagulate the **latex.** Care should be taken to place the material in alcohol **directly it is cut,** or at least the cut surfaces should be wetted with alcohol so as to check the flow of latex from them. If the latex be allowed to escape, the laticiferous tissues are emptied, and are then much less easily traced than when they are full. The best method is perhaps to preserve the **whole plant** without injury in alcohol, in which case the latex will not be lost at all.

Draw from a piece of the fresh stem of *Euphorbia* a drop of latex upon a slide: examine it quickly under the microscope, and observe the fluid is at first almost uniformly milky, but that in a short time a **coagulum** separates in irregular masses from the more transparent fluid. The coagulation is effected more completely and rapidly on addition of a drop of alcohol.

i. *Laticiferous Vessels.*

I. Cut tangential sections from the phloem of the root of the Dandelion (*Taraxacum officinale*), mount in potash and glycerine, and warm; examine under a low power.

The main constituents of the tissues are parenchy-

matous cells, with thin walls (**phloem-parenchyma**): **sieve-tubes** are to be met with here and there. The whole mass of tissue is permeated by a ramifying, and profusely anastomosing network of **laticiferous vessels.** The communication of these tubes with one another is demonstrated by the continuity of their coagulated contents (**latex**), which appear brown and granular.

The course of the vessels is mainly longitudinal, while lateral, horizontal branches frequently connect the parallel tubes.

With a high power make out more accurately the course of a group of the vessels.

By staining other sections with alkannin (Appendix A), or with solution of potassium bichromate, good preparations may be obtained.

II. Cut transverse sections of the same; mount in glycerine, and examine with a low power.

The laticiferous vessels appear circular in transverse section, and have brown contents: they are distributed in groups, which form more or less regular concentric rings round the central xylem. They may be recognized still more distinctly in sections stained with alkannin, or with potassium bichromate.

Note in these sections the presence of **sphere-crystals** of **inulin**: in the former section they will have been dissolved by the treatment with potash. Observe that they are formed quite irrespective of the cell-walls, which are often included in them.

The development of the laticiferous vessels may be traced by cutting thin longitudinal sections through the cambium of the root of the Dandelion. By careful comparison of such sections it

will be found that they originate from a number of originally separate cells of the cambium, the cavities of which are thrown together by the partial or complete absorption of the walls. Such fusions may appear in the terminal or the lateral walls.

Note on Inulin.

From a fresh tuber of the Jerusalem Artichoke (*Helianthus tuberosus*), or from fresh Dandelion roots cut sections in any direction, and mount them in a very small quantity of water: examine under a low power, and observe that the tissue is chiefly composed of cells with transparent contents.

Irrigate the sections well with alcohol: a granular precipitate will appear in the fluid surrounding the sections, and a similar precipitate will also be seen within the cells, which may be so bulky as to make the whole section appear opaque.

Now irrigate thoroughly with water: the precipitate will again dissolve.

Cut sections from material which has been kept for some weeks, or better for some months, in alcohol: mount in glycerine, and observe the transparent tissue as before: here and there will be seen large circular patches of highly refractive substance, which are of such size and position as often to extend over a number of cells: these are the **sphere-crystals of inulin.**

Treat with iodine solution: they are not appreciably coloured. Irrigate well with water: they are slowly dissolved, their solution being hastened by warming, and they show a radiate structure as solution progresses. Treat with potash: they are dissolved more quickly, without any coloration.

A similar substance, **hesperidin,** is found in young Oranges: if these be treated for a long time with alcohol, the tissues will be found crowded with sphere-crystals, which have similar reactions to those of inulin, but on dissolving in potash the solution takes a yellow colour.

ii. *Laticiferous Cells.*

I. Cut tangential sections of the cortex of *Euphorbia splendens* (other species will do) just outside the vascular

ring, and mount in water, or dilute glycerine: or stain with alkannin, and mount in glycerine.

Examine with a low power.

Running through the cortical parenchyma will be seen long tubes, with thick cellulose walls and granular contents. These are the **laticiferous cells,** which differ from the preceding in being developed, not by fusion of originally distinct cells, but by continued apical growth of single cells.

Note cases of **branching** of these cells.

Included in the granular contents are **starch-grains** of peculiar dumb-bell form.

Treat sections with iodine solution, and observe the effect on these bodies.

II. Cut transverse sections of the same stem, and note the distribution of the laticiferous cells; they may be recognized by their walls, which are thicker than those of the surrounding tissues, and appear circular in section.

III. Separate the whole cortex from a piece of the stem; boil it in potash for about five minutes, and tease out the long laticiferous cells with needles; mount, and observe with a low power. They appear as long cylindrical tubes, with thick walls. Observe occasional branching. They are usually broken at the ends, the length of the tubes being greater than that of the parts teased out.

In longitudinal sections through the apical region of the stem of *Euphorbia* it may be shown by staining with hæmatoxylin that numerous nuclei are present near to the blind endings of the tubes.

LEAF

i. *The Common Bifacial type*

A.—PETIOLE

Observe that the mature leaf of the Sunflower consists of an upper, flat, expanded portion—the **lamina,** and a lower, narrow stalk—the **petiole,** by which it is inserted on the stem. Note the channelled upper surface of the petiole, and the broad insertion on the stem: in the angle between the petiole and the stem may usually be observed an **axillary bud,** or **shoot.**

I. Cut transverse sections of the petiole, and mount in glycerine. The details of structure resemble in many respects those of the young stem, from which the petiole differs in the following points:—

1. The general outline of the section is semilunar, the concave being the superior, while the convex is the inferior surface: thus the petiole is **dorsi-ventral,** whilst the stem is **polysymmetrical.** This property extends also to the arrangement of the vascular bundles, of which the xylem is as a rule directed towards the upper surface of the petiole.

2. In the presence of numerous **stomata;** beneath each stoma the collenchyma is replaced by chloro-

phyll-containing parenchyma with intercellular spaces Note beneath each stoma an enlarged intercellular space—the air-chamber.

3. In the number and arrangement of the vascular bundles. In the petiole there are three main bundles, besides several smaller ones (compare the rough dissection of the stem, p. 56).

4. In the absence of interfascicular cambium: the larger bundles are, for a short time at least, open bundles, having an active cambium, while the smaller ones are closed, having no secondary thickening by cambium.

5. No general bundle-sheath is present, though each bundle is surrounded by a layer of colourless cells without intercellular spaces, which may be regarded as representing the bundle-sheath.

On the petiole of some leaves, both of Dicotyledons and Monocotyledons, a cylindrical swelling or **pulvinus** is found: it is usually at the base of the petiole, and smaller ones may also be found at the foot of each pinna: this may be clearly seen in *Phaseolus*, and other Leguminosæ. Leaves provided with a pulvinus are frequently capable of change of position, turning upon it as upon a hinge: observe the difference of position of the leaves of *Phaseolus* at midday, and in the evening.

Cut transverse sections through the pulvinus of *Phaseolus:* mount in glycerine or chlor-zinc-iodine, and observe—

1. The **epidermis,** with a well-marked cuticle, and conical, often hooked hairs.

2. A broad band of **cortex,** consisting of cells with thin cellulose walls, and very small intercellular spaces.

3. This is limited internally by a **starch-sheath,** which surrounds a central cylindrical vascular mass: this is composed of—

4. A peripheral band of **phloem,** with considerable collenchymatous thickening of the walls of the outer portion.

5. Internally are radiating rows of vessels of **xylem**, separated by rays of tissue, which, together with the central pith, show a collenchymatous thickening of the cellulose walls.

Cut successive transverse sections at the upper limit of the pulvinus, and on into the normal petiole, and note how the central vascular mass separates into distinct bundles, forming an irregular ring round the central, thin-walled or even fistular pith. The structure of the pulvinus is thus well adapted to its function as a hinge, or organ of movement.

B.—LAMINA.

I. Take a piece of the lamina of the leaf of the Sunflower, including the apex: it is important that it should be previously bleached by treatment with alcohol: warm it gently in a mixture of dilute glycerine and potash, and mount in glycerine: examine with a low power, and observe—

1. The **midrib**, with its strongly marked **vascular bundle**, running up to the apex of the leaf, where it terminates abruptly in a mass of glandular parenchymatous tissue.

2. Lateral **branch-bundles**—the **ribs** or **nerves**—passing off from it, and forming a network by frequent anastomoses, while some of them run up into and terminate in the serrate projections of the margin of the lamina in a manner similar to the midrib as above described.

3. Smaller branch-bundles, sometimes showing **blind endings** in the parenchyma which fills the meshes of the network.

Vascular skeletons of the simple, coriaceous leaf of *Buxus sempervirens* may be prepared, so as to show the entire network,

by boiling for ten minutes in 10 per cent. solution of potash ; then by means of forceps, and a stiff camel's-hair brush, the superficial tissues and mesophyll may be removed, leaving the vascular tissue as a continuous network, limited at the periphery by a larger marginal bundle : mount the skeleton thus prepared on a slide, and examine under low power : note especially (1) the branchings and fusions of the ribs or nerves ; (2) the blind endings of the smaller ones ; (3) the large peripheral bundle ; (4) cells of the mesophyll still adhering to the bundles of the network, and partially filling up the interstices.

Various other leaves may with advantage be treated in a similar manner, or observations may be made upon leaves of poplar, &c., of which the softer tissues have rotted by lying on damp soil, while the vascular system remains as a skeleton showing often with great perfection the arrangement of the vascular network.

II. Cut off a small square piece of the lamina of a leaf of *Helianthus*, including one of the main **ribs** or **nerves,** and embed in paraffin (see directions, p. 11), so that the rib shall be perpendicular. Cut transverse sections, and mount in glycerine. If cocoa-butter has been used, it may be dissolved off the sections with ether or chloroform.

Good sections may be obtained even from fresh material by holding the piece of lamina between slices of carrot, or pith ; or by folding the whole lamina repeatedly, and cutting sections from the whole mass. In these cases, though the chlorophyll appears of a better colour, the sections not having been treated with a solvent (alcohol), still they will be infested with air-bubbles, which may be partially removed by leaving the sections for some minutes in water : they may be completely removed (though the chlorophyll would be dissolved) by more prolonged treatment with alcohol. Difficulty will often be found in obtaining good preparations of the above : all the important points may be more easily observed in the leaves to be described below.

Note with a low power—

1. The general outline of the section, which is irregular and undulating, though it is in the main of uniform breadth. At the point corresponding to the main nerve the section widens out, the nerve appearing **semilunar,** as in the petiole. The convex side is the inferior (**anterior** or **dorsal**), and the concave the superior (**posterior** or **ventral**) surface.

2. That the margins of the sections (*i.e.* the superior and inferior surfaces of the leaf) are studded with projecting **multicellular hairs.**

3. That the arrangement of the tissues in the large nerve resembles that in the petiole, though less complicated. Thus it often has but one **large central bundle,** with smaller lateral ones. The position of the xylem and phloem relatively to the whole leaf corresponds to that in the petiole, *i.e.* xylem towards the upper surface, phloem towards the lower.

Occasionally some of the smaller bundles in the vein are inverted, showing an approach to the arrangement of bundles in the polysymmetrical stem.

4. **Smaller veins,** with correspondingly reduced vascular bundles, are found scattered through the thinner part of the section.

Next examine the thinner part of the section with a high power, and, starting the study of them from the upper surface, note successively the following tissues:—

1. Upper layer of **epidermis,** continuous with that covering the nerve: it is a single layer of cells, covered externally by **cuticle,** which has the same characters as that of the stem. The epidermis bears numerous

multicellular **hairs,** already noted in connection with the apical bud. **Stomata** occur in considerable numbers. Beneath this layer lie—

2. Thin-walled, oblong cells, with copious protoplasm, and **chlorophyll-grains**; they are arranged with the longer axis perpendicular to the outer surface, and form two layers; this tissue, from the form and arrangement of the cells, is called the **palisade-parenchyma**; below it is—

3. A mass of parenchymatous cells of irregular form, with large intercellular spaces; in general characters they resemble (2): this is the **spongy parenchyma.**

(2) and (3) are together included under the general term **mesophyll.** Embedded between (2) and (3) are—

4. Numerous smaller vascular bundles (**nerves**) of various size, often reduced to a single pitted or spiral tracheide, surrounded by a colourless **sheath** of parenchyma similar to those in the petiole. The course of these bundles is diverse, since they form the reticulate system of veins: they may thus be seen in the sections to have been cut transversely, obliquely, or longitudinally.

5. A second layer of **epidermis** bounds the section on the lower side; it has the same characters as the upper layer, but stomata are more frequent. Note the two small **guard-cells** of each stoma, and below them the large **air-chamber.**

Hairs as before seen on the upper surface. Note the mucilaginous walls of these hairs.

The structure of the leaf of the Sunflower is an ordinary type for herbaceous plants: compare with it, by means of similar sections, that of the leaf of the Wallflower (*Cheiranthus Cheiri*): the

general arrangement of the tissues will be found the same, but there are differences of detail, *e.g.* unicellular hairs of peculiar spindle-form are found on both surfaces, there are three or more layers of palisade-parenchyma, &c.

Sections may also be cut with advantage from the leaf of the *Dahlia.*

Note on Chlorophyll Corpuscles, or Chloroplasts, and Chlorophyll.

These have already been described on p. 47 : the characters of form, mode of division, and solubility of the green colouring matter (chlorophyll) there noted should be verified on the chloroplasts of the Sunflower, or some other leaf. Chloroplasts which have been bleached by alcohol should be stained with iodine, when they will assume a brownish tint.

If chlorophyll-corpuscles, which have been treated with picric acid and decolorized with alcohol, be stained with iodine, Hoffmann's blue, or hæmatoxylin, and be examined with a very high power, it will be seen that they have a trabecular structure. The leaves of *Vallisneria* afford good material for this observation. The minute structure of the corpuscles can also be readily made out in cells of the leaves of *Echeveria.*

Solution of Chlorophyll in Alcohol.—In order to prepare this, a quantity of fresh grass or of cabbage-leaves is to be taken, and freed as far as possible from decayed leaves ; it is then to be boiled in water, pressed so as to get rid of as much water as possible, and spread out on a sheet of paper to dry in a dark place ; when dry it is to be soaked in alcohol for some hours in a dark place. When the alcohol is poured off and filtered, it will be found to be deeply coloured, owing to the solution of the colouring matter (chlorophyll), while the mass of leaves will be seen to be bleached.

Take a small quantity of the solution in a test-tube, and examine it first by light transmitted through it (*i.e.* hold it between the window and the eye) : it will appear of a deep green colour. Examine it now by reflected light (*i.e.* the observer is to place himself between the window and the tube, so that his eye receives light reflected from the tube), the solution will appear a deep dull red : the solution is thus *dichroic.*

The following is a convenient mode of examining the solution spectroscopically: the tube of a microscope is withdrawn (this may be easily done with the smaller forms of Zeiss's, Hartnack's, and Crouch's microscopes), and it is replaced by a glass tube, the bottom of which covers the opening of the stage of the microscope; the sides of the tube must be made opaque by wrapping round them a sheet of black paper; the solution is then poured into the tube, and into the opening of the tube a microspectroscope is introduced; the mirror of the microscope is to be so inclined that it reflects a beam of light on to the bottom of the tube. The advantage of this method is that it enables the observer to vary the thickness of the layer of the solution to be examined, and this has its effect on the appearance of the spectrum, as will now be shown.

It is best to use a dilute alcoholic solution. Beginning with a column of the solution about $\frac{3}{4}$ of an inch in height, the spectrum will present a single rather narrow absorption band (band I.), in the red, about the line *C* of the solar spectrum, extending towards *B*; if the height of the column be about doubled, band I. will be seen to have become broader, a faint narrow band (band II.) will be seen to the right of it, between the lines *C* and *D*, at the beginning of the orange, another faint narrow band (band IV.) in the green a little to the left of the line *E*, a broad faint band (band V.) in the blue to the right of the line *F*, a still broader faint band (band VI.) in the blue and indigo just to the left of the line *G*, and finally a broad faint band (band VII.) at the extreme violet end of the spectrum. On increasing the height of the column to about six inches, the bands I., II., IV. will be seen to have become broader and darker, and the bands V., VI., VII. to have coalesced so as completely to cut off the spectrum to the right of the line *F* in the blue; a new band (band III.), rather broad but faint, will be seen at the junction of the yellow and of the green a little to the right of the line *D*.

By this means it is possible to ascertain that the spectrum of chlorophyll presents seven distinct absorption bands.

Included Starch-grains.—These may be observed in the cells of the mesophyll of any leaf which has

been exposed to the light, under conditions suitable for assimilation: but they may be seen with special ease in Fern prothalli which have been thus exposed to bright sunlight for some hours, and then bleached in alcohol.

Mount such a bleached prothallus in water, or in weak glycerine: examine under a high power, and note the bleached chlorophyll-corpuscles, or **chloroplasts,** in which highly refractive granules may often be seen.

a. Stain with iodine solution: the chloroplasts will assume a dusky bluish colour, the blue tint being more or less distinctly localized in the highly refractive granules (**starch-grains**) above noted.

b. The presence of the included starch-grains may be more clearly demonstrated by causing them to swell: this may be effected in various ways.

i. Mount in glycerine and iodine, and warm: the high temperature will swell the starch, which will at the same time stain with the iodine.

ii. Treat with potash, and after carefully washing out the alkali stain with iodine.

iii. The best method is, however, that introduced by Schimper. Treat the bleached specimens for some hours with a solution of iodine in chloral hydrate: the included starch-grains are simultaneously swollen and stained blue.

III. Since the structure of the leaf of the Sunflower is not a universal type, it will be well to study also the structure of some other leaves; for instance, the evergreen leaves of the Holly (*Ilex Aquifolium*): note first the short, almost cylindrical petiole, and the leathery lamina with cartilaginous margin and spines.

Cut transverse sections of the petiole of the Holly: mount in glycerine, and note in the slightly oval section under a low power the epidermis, and broad band of cortex: also the large, semilunar vascular bundle which occupies a central position, and has its xylem disposed at the concave side, nearer to the upper surface, and its phloem disposed at the convex side, which is directed towards the lower surface of the petiole. One or two small outlying bundles are also to be seen.

Note that there is a cambium-layer, which shows signs of activity, between the xylem and phloem of the large bundle.

IV. Transverse sections of the lamina of the Holly may be prepared as above directed for the Sunflower, and be mounted in dilute glycerine, others in chlor-zinc-iodine. Starting from the upper surface of the thin lateral portion of the lamina, observe successively the following tissues:—

1. **Epidermis,** a single even layer of cells, with thick walls, and colourless protoplasmic contents; no hairs or stomata are to be seen; the lateral walls are pitted: note in sections treated with chlor-zinc-iodine that the outer wall, which is thicker than the rest, is differentiated into—

a. **Cuticle,** a continuous, well-defined and highly-refractive layer, covering the whole epidermis externally: this stains yellow with chlor-zinc-iodine.

b. **Cuticularized layers,** of granular appearance, and stained a deeper colour than (*a*) with chlor-zinc-iodine: they are intermediate in properties between cuticle and true cellulose.

c. The **cellulose-layer,** which abuts on the cavities of the cells: this stains blue with chlor-zinc-iodine.

i. These several layers are not easily distinguished in preparations simply mounted in glycerine, but may be readily distinguished in sections treated with a weak solution of fuchsin: *a* and *b* stain much more readily than *c*.

ii. Treat sections with concentrated sulphuric acid. *a* retains a sharp contour; the rest of the wall swells, and loses distinctness of outline.

iii. Boil some sections for a long time with strong potash. *a* and the cuticular granules of *b* will be dissolved, while *c* and the cellulose matrix of *b* will remain.

2. **Hypoderma,** a strengthening tissue, which is immediately below the epidermis, and consists of a single layer of cells: at the midrib it may widen into two layers: the walls are pitted, and stain bluish with chlor-zinc-iodine.

3. The **palisade-parenchyma,** composed of thin-walled, oblong, closely-packed cells, with their longer axes perpendicular to the surface of the leaf; the cells are somewhat irregularly arranged in three layers; observe **nuclei** and **chlorophyll-grains;** here and there are cells (**idioblasts**) with but little protoplasm, in which is inclosed a large crystal. Passing towards the lower surface of the leaf, this tissue merges gradually into—

4. The **spongy parenchyma,** the cells of which resemble those of (3) in general characters; but their shape is various, and large intercellular spaces occur. Idioblasts with crystals are scattered here and there. Embedded between (3) and (4) are—

5. **Vascular bundles** of various size; the direction in which these run is not uniform, as is naturally the

case, since the venation is reticulate: the positions of xylem and phloem with regard to the whole leaf are the same as in the Sunflower: the bundles are surrounded by a continuous colourless sheath of cells (the bundle-sheath or **endodermis**) without intercellular spaces. At the lower limit of the section lies—

6. The lower **epidermis,** which resembles (1) in general character; but differs in having numerous **stomata.** Note the appearance presented where the two **guard-cells** of a stoma have been cut transversely, and observe carefully—

a. The form and position of the two **guard-cells.**

b. The cavity or intercellular space between them (the **pore**): this leads into

c. The large, intercellular space (**air cavity**) in the tissue beneath the stoma.

d. In the sections stained with chlor-zinc-iodine or with fuchsin, note the continuity of the cuticle round the guard-cells, into the pore of the stoma.

The stomata are small in this leaf, as in most of the Dicotyledons, and it is consequently somewhat difficult to make out the details of their structure: for examples of larger and more easily observed stomata, and the observation of the details of their structure, reference should be made to the work dealing with the Monocotyledons (p. 175, &c.), in which the stomata are often of considerable size.

V. Cut tangential sections from the upper and under surfaces of the leaf of the Holly, and mount them separately in glycerine with the external surface in both cases uppermost.

The cells of the **upper epidermis** are tabular, with irregular outline; the surface has a granular appearance,

explained by the granular cuticularized layers observed in transverse sections; the lateral walls are pitted, the contents colourless, and there are **no stomata.**

The cells of the **lower epidermis** are similar to the above; but **stomata are numerous;** they have no definite arrangement. Note the two sausage-shaped nucleated guard-cells, inclosing the pore; they contain chlorophyll, and are slightly depressed below the general surface.

VI. Cut transverse sections of the cartilaginous margin of the leaf of the Holly, and observe the same structure as before; but towards the rounded margin the hypoderma increases in thickness to two or more layers, and at the extreme margin a large mass of sclerenchyma lies immediately below it: the cells have thick stratified and pitted walls: they give the reactions of lignified walls but not distinctly or readily. This tissue is of importance, as giving strength and rigidity to the leaf.

N.B.—The Cherry Laurel (*Prunus Lauro-Cerasus*) may be used instead of the Holly, the structure being very similar, with the exception that the hypoderma and marginal sclerenchyma are absent.

ii. *Isobilateral Type.*

For comparison with the above, which is by far the commonest type, examine the laterally compressed petiole (**phyllode**) of some one of the phyllodineous *Acacias, e.g. A. armata* or *A. heterophylla:* observe in the latter species that the petiole of some leaves which

bear a bipinnate lamina, is flattened in a vertical plane, while in other cases where the petiole is larger, the lamina is almost entirely absent: the latter is constantly the case in *A. armata*. Note the reticulate venation.

I. Cut transverse sections through a phyllode, mount as before, and observe that the tissues are arranged symmetrically with reference to the two flat surfaces: thus—

1. **Stomata** are found in the epidermal layer of both sides.

2. **Palisade-parenchyma** is found immediately below both epidermal layers. There may (*A. heterophylla*), or may not (*A. armata*) be a central mass of colourless tissue.

3. **Vascular bundles** are found with their phloem and sclerenchyma directed towards the outer surface, and the xylem towards the centre: these bundles are frequently opposite one another, and with their respective masses of xylem in close proximity: compare the girder-like arrangement of the vascular bundles and sclerenchyma so common in the leaves of Monocotyledons. Note also a single vascular bundle, strengthened with sclerenchyma at both the upper and lower edges of the compressed petiole. Compare the leaf of *Iris* (see below, p. 179).

II. An actual transition from the bifacial to the isobilateral type of structure of the leaf may be traced in *Eucalyptus globulus* and other species, in which the lamina of the lower leaves is extended in a horizontal plane, the upper leaves of the plant in a vertical planes: the former show two characteristic layers of palisade-parenchyma below the upper epidermis, while the rest

of the mesophyll is a characteristic spongy parenchyma.

If tangential sections be cut, it will be found that there are no stomata in the upper epidermis, but large numbers on the lower.

If transverse sections be cut from one of the upper, vertical leaves, it will be found that below the epidermis of either side there is palisade-parenchyma: stomata are also found in both epidermal layers; but the vascular bundles retain the position with the xylem directed towards the morphologically upper surface. Note the large lysigenetic oil-cavities.

Similar oil-cavities are to be found in *Ruta*, *Citrus*, &c. Compare also the intra-mural glands in the leaf of *Psoralea*. Examine young specimens of the above, and trace their development.

iii. *Centric Type.*

The leaves above examined are of distinctly flattened form; in leaves of another type, called the *centric* type, the distinction of an upper and lower surface is but very slightly marked, and the whole leaf is of approximately cylindrical form. It is usually in leaves of succulent plants that this arrangement is found, and as an example we may take the leaf of *Sedum acre* (the common Stonecrop); the petiole is entirely absent in this simple form of leaf.

I. Cut transverse sections of the fresh leaf of the Stonecrop; mount in water, or dilute glycerine, and observe that the **outline** of the section is even and oval: the arrangement of tissues is concentric, and is

uniform all round, so that beginning at any point of the periphery and passing inwards we encounter—

1. The **epidermis,** a single layer of cells of variable size and shape, with well-defined **cuticle,** and **stomata,** the guard-cells of which are much smaller than the epidermal cells.

2. **Chlorophyll-containing mesophyll,** which is not differentiated into palisade and spongy parenchyma: this tissue forms the great mass of the leaf; intercellular spaces occur: the cells are thin-walled, with a protoplasmic sac, in which are embedded **chlorophyll-grains,** and there is a large central vacuole. Observe the chlorophyll-grains undergoing division. Embedded in this tissue lie centrally—

3. **Vascular bundles** of small size: their number varies from 3 to 5.

II. Strip off a piece of **epidermis** from the leaf of *Sedum acre,* and mount in water. Note—

1. The nucleated **epidermal cells** with sinuous outline; they contain no chlorophyll.

2. The **stomata** with two guard-cells surrounding the pore, as in the Holly, or Cherry Laurel. Surrounding these are—

3. Three **subsidiary cells,** which differ in size and shape from the ordinary epidermal cells, and are arranged in definite order round each stoma.

Beneath the epidermis will usually be found cells of the **mesophyll,** with thin walls, large vacuole and protoplasmic sac, in which are embedded **chlorophyll-grains.**

By making similar preparations from successively younger leaves the development of the stoma and subsidiary cells may be

traced as follows. From one of the epidermal cells a smaller cell is cut off; from this are successively cut off the three subsidiary cells: the remaining cell is the mother-cell of the stoma, which divides to form the two guard-cells.

iv. *Aquatic Type.*

Note the difference in form between the submerged and aërial leaves of *Hippuris:* the former are long, very thin, especially at the margins, and of a pale green colour; the aërial leaves are shorter, more bulky, and of a deep green colour.

I. Cut transverse sections of the aërial leaves, either from fresh or alcohol material: mount in glycerine, and observe—

i. The oval form of the section.

ii. The clearly defined **epidermis,** with a well-marked **cuticle; stomata** are found on both surfaces, also peculiar **disk-like hairs,** which will here be seen cut through vertically, and are inserted in the epidermis by a unicellular pedicel.

iii. The **mesophyll,** consisting of a lax palisade tissue towards the upper surface, and still more lax spongy parenchyma towards the lower.

iv. **Vascular bundles,** each sharply circumscribed by a bundle-sheath: one large bundle occupies the centre, and smaller bundles are disposed laterally.

II. Cut similar sections of submerged leaves, and note that—

i. The section is much narrower, but more extended.

ii. The epidermis is clearly marked, but **stomata are**

absent from both surfaces : discoid hairs are occasionally seen.

iii. The mesophyll, which is much less bulky, and is reduced to a single layer of cells in the marginal parts, is not differentiated into palisade and spongy parenchyma.

iv. Only one central vascular bundle is present.

The absence of stomata should be confirmed by tangential sections from both upper and lower surfaces : at the same time note the discoid hairs in surface view.

A comparison, in respect of the distribution of stomata, may be made with floating leaves, *e.g.* of *Nymphæa* or *Nuphar*, in which stomata will be found on the upper surface which is exposed to the air, but not on the lower submerged surface.

I. **Multiple epidermis.** In not a few leaves the epidermis (derived from the single layer of dermatogen) consists of more than a single layer of cells, owing to periclinal divisions of the cells of the dermatogen : this multiple epidermis is sometimes styled aqueous tissue.

Cut sections of the lamina of various species of *Begonia*, and note the narrow green band of mesophyll, bounded above and below by bands of transparent aqueous tissue, the number of layers varying in different species, and the upper bands being broader.

Similar observations should be made on leaves of species of *Peperomia*, in which, as in *Begonia*, the upper band of aqueous tissue is the broader, extending even to fifteen or sixteen layers of cells in some species.

Sections should also be cut of the lamina of *Ficus elastica* (the India-rubber Plant), or other species : note the three rather irregular layers of colourless tissue composing the upper epidermal band, the outermost consisting of the smallest cells : also the large cells with cystoliths : the narrower band of lower epidermis, con-

sisting of three layers, with depressed stomata: also the central mesophyll, differentiated into palisade and spongy parenchyma.

Sections should be cut from young leaves, from which evidence may be obtained that the aqueous tissue is really the product of periclinal division of the originally simple layer of dermatogen.

The bodies known as **cystoliths** are the most prominent deposits of calcium carbonate to be found in connection with cell-walls. In order to see these mount transverse sections of a leaf of *Ficus elastica* in water, and examine with a high power.

Observe the layer of large clear cells underlying the superficial layer of the epidermis of the upper surface of the leaf: here and there one of these cells is seen to contain a botryoidal body suspended by a stalk from the top of the cell; this is a cystolith: it consists of a mass of cellulose developed as an outgrowth from the cell-wall, incrusted with calcium carbonate.

Run in a drop of acetic acid: observe that the cystolith becomes gradually transparent, and that an evolution of bubbles of gas (CO_2) is taking place from it.

When the calcium carbonate is all dissolved, a mass of cellulose will be seen to remain, presenting both striation (perpendicular to its margin) and stratification (parallel with its margin). Apply tests for cellulose to this residue.

II. **Mechanical strengthening of leaves.** The sclerenchyma (stereom) has been above alluded to in the case of the lamina of the Holly, and the phyllode of *Acacia*, as a tissue which is useful in giving strength and rigidity to the other less firm tissues: a further and striking example of the distribution of sclerenchyma so as to attain this end is seen in species of *Hakea* —for instance, in *H. suaveoleus*. Cut transverse sections of the very rigid pinna, and, mounting as before, note that the oval section is limited by a firm layer of epidermis, with here and there stomata depressed below the general surface, and bounded by two very small guard-cells, with a pair of small subsidiary cells on either side: below this are two layers of palisade parenchyma, and centrally a colourless mass of parenchyma inclosing several vascular bundles, and scattered sclerenchymatous elements. Note especially the large, sometimes branched, sclerenchymatous cells, which extend from the epidermis to the central mass of

tissue, and constitute a firm framework, the interstices of which are filled with the thin-walled parenchyma.

Compare also the tissue of the mesophyll in the lamina of *Camellia* and *Hoya*, throughout which sclerenchymatous elements are irregularly distributed.

III. **Water-stomata and marginal glands.** Examine leaves of the *Fuchsia*, and observe that the tips of the leaves and the marginal teeth are terminated by slightly swollen, opaque masses of tissue: these are the marginal glands, and drops of water exuded at those points may be seen in the morning, or on plants kept during the day in moist atmosphere at a high temperature.

Cut off a piece of the margin of a leaf and examine it under a low power: a large stoma (**water-stoma**) may be recognized at the apex of each tooth: below it is a pad of opaque tissue (the gland) to which a vascular bundle runs up, and in which it ends.

From material which has been hardened in alcohol, cut off with a razor the extreme tips of several of these teeth: mount with the outer surface uppermost: on examining these, the extreme apex, with the water-stoma, will be seen in surface view. Note the size of the stoma, and that the pore is widely expanded: a *camera lucida* drawing of the water-stoma should be made, and compared with a similar drawing of an ordinary stoma from the same leaf: it will then be seen that the water-stoma is of larger size.

Cut longitudinal sections so as to follow the vascular bundle up to the marginal gland, and to traverse the gland in a median plane. Selecting a section which is really median, note, in the part of it further from the tip, the epidermis, mesophyll, and the vascular bundle surrounded by a parenchymatous sheath: following these up towards the tip, observe th eepidermis continuous, with the exception of the widely-gaping water-stoma at the extreme apex: the form of the guard-cells as seen in section is simpler than in ordinary stomata. The vascular bundle widens out towards the tip, and the vascular elements terminate in the pad of closely packed parenchyma of the gland (**epithema**): there is a large cavity below the water-stoma.

Similar observations should be made at the points of exudation of water in leaves of *Primula sinensis*, *Tropæolum*, &c.

Special attention should be paid to the **chalk-glands** of the

Saxifragaceæ (*e.g. Saxifraga crustata*) in which the structure of the marginal gland is extremely well seen, while the accretions of chalk deposited by the evaporating water are easily recognized with the naked eye. *S. oppositifolia* or *S. umbrosa* (London Pride) will afford excellent material for the study of these glands. Compare also various *Crassulaceæ*.

Treat some of the accretions with acetic acid, and note their solution with evolution of bubbles of CO_2.

IV. **Extra-floral nectaries.** Examine the back of the leaf of the Cherry Laurel, and near the base of the lamina, on either side of the midrib, will be seen circular areas, of variable number, and with a smooth surface and opaque appearance: these are **glands**, which produce a sugary secretion.

Cut transverse sections so as to traverse one or more of the glands: mount some in glycerine, others in chlor-zinc-iodine: observe the transition from the ordinary epidermis of the lower surface with the thick outer wall, to the **glandular epithelium** consisting of cells which are narrow and deep, with plentiful granular protoplasm and nucleus; the lateral walls are thin; the external wall is rather thicker and covered by a continuous cuticle (as shown on treatment with chlor-zinc-iodine), which may however be separated from the wall as a thin film, and be ruptured. Note also a band of cells with peculiarly thickened walls surrounding the margin of the glandular spot.

Below the epithelium is a mass of parenchyma without intercellular spaces: the walls are slightly thickened and pitted, and the cells have plentiful protoplasm: this tissue merges gradually into the ordinary mesophyll. Below the glands, and closely connected with them, vascular bundles will be seen.

Sections may also be made through similar glands on the leaves of other species of *Prunus*, or of *Viburnum Opulus*: the dark spots on the under surface of the stipules of *Vicia* show a different structure, the epidermis there giving rise to numerous capitate hairs: a sugary secretion is exuded in some species.

Sections may also be made of the pocket-shaped glands on the involucre of *Poinsettia*, previously hardened in alcohol. Cut them longitudinally, and mount in glycerine; or, better, stain with Kleinenberg's hæmatoxylin, and mount in Canada balsam.

Observe, under a low power, the deep and narrow pocket lined with an epithelial layer, which gets deeper towards the base, while vascular bundles form an irregular plexus below the base of the pocket.

Starting from the outer lip of the gland, examine the superficial lining under a high power, and observe that there the epidermis is a simple layer of cells of no great depth, and with a thickened outer wall: passing down the inner surface of the pocket, the cells become gradually deeper and narrower, and occasional periclinal divisions may be seen, till at the base the **glandular epithelium** may consist of three irregular layers of cells, with thin cell-walls, dense protoplasmic contents and nucleus.

Treat another section with a drop of sulphuric acid: the superficial **cuticle** resists the acid.

The subjacent tissue may be examined for ends of the laticiferous tubes, in which a number of nuclei may be found in the stained sections.

Sections should also be cut through **nectaries** of various flowers: species of *Fritillaria* will be found to be good material for this work.

Leaf-scars and Fall of the Leaf.

On twigs of the Elm cut in winter, note the buds, both terminal and lateral, and below each an oval *scar*, which indicates the surface of separation of a leaf when it fell in autumn: the surface of the scar is brown, and the slightly projecting dots upon it are the broken ends of the vascular bundles which ran out from the stem into the petiole.

Cut longitudinal sections so as to pass through a scar, and select for observation one of those which has followed up the course of one of the vascular bundles to

the surface of the scar: mount in glycerine, and observe below the scar the tissues as above described for the stem (p. 88 &c.). At the level of the scar the following structural points are to be noted:—

1. The rough and irregular outer limit of the tissues, with dried up remains of cells often projecting beyond the general surface.

2. The dark brown band of **cork,** without intercellular spaces, which covers the scar, and protects the internal tissues.

3. The projecting end of the vascular bundle: mark especially how the corky formation is continuous into the tissues of the bundle, evidently having there arisen from the division of cells of the parenchyma of the bundle: also note that the vessels of the xylem are laterally compressed by the adjoining cells, and their broken ends are thus closed at the surface of the scar.

Sections should also be made from material taken in autumn just at the period of the fall of the leaf, so as to see the changes in the tissues at the base of the petiole which precede the rupture. These may be particularly well seen in the Horse-Chestnut. In longitudinal sections through the base of a leaf, which is almost ready to fall, note that the brown band of cork on the stem stops short at the base of the leaf, and that close to the point where it stops is the starting-point for the similar corky layer which runs directly across the base of the petiole, cutting the vascular bundles at right angles, and continuous through them.

Examine such a section under a high power, and note—

1. The radial rows of cork-cells, with the phellogen on the side next the stem.

2. A band of cells outside the cork, which are of yellowish colour, and the individual cells are rounded, having large intercellular spaces between them; thus this layer, which has been styled the **abciss layer,** is easily ruptured.

3. The cells of the tissues of the leaf are almost empty with the exception of crystals: those of the stem, below the layer of cork, have plentiful protoplasm, and starch.

Comparisons may also be made of similar material of the Poplar, Ash, or Walnut, which are well adapted for illustrating the process of defoliation.

HAIRS AND EMERGENCES.

Hairs and emergences have already been observed on the stems and leaves above described: as there is in different plants great variety in the form and structure of these outgrowths, a series of characteristic types will now be mentioned as suitable for observation.

Hairs.

1. *Simple and unicellular.* On the stem of the Elm these have been seen of **conical** form (p. 90): **filiform** unicellular hairs are seen, as root-hairs on most roots, *e.g. Trianea* (p. 33), and below (p. 150) in *Phaseolus*, &c.: **cottony** hairs may be well seen on the ripe seed of *Epilobium*, when mounted in water, after wetting with alcohol. Larger forms of the simple unicellular hair are often seated on an emergence; *e.g.* the **stinging hairs** of the Nettle: cut transverse sections of the stem or petiole of the Nettle, taking care not to injure the hairs in the manipulation: mount in water: note the large, conical, unicellular hairs, with obliquely bent apex each being seated on a multicellular foot or emergence.

2. *Multicellular hairs*, of *conical form*, and divided by septa, are found in the Scarlet Geranium: cut transverse sections of the petiole or stem, and observe these hairs together with capitate hairs. Hairs of a similar type, but more bulky form, have already been seen in *Helianthus* (p. 62). Some of the hairs of the Scarlet Geranium may be seen to be *branched*, and this is often constantly the case in other plants: as an example of a very simple branching, cut longitudinal sections of the leaf or stem of the Wallflower, and note the unicellular, *spindle-shaped* hairs: more profusely branched hairs are found on the surface of the stem or leaf of species of *Verbascum:* it will be found better to take one of the less woolly species, *e.g. V. nigrum.*

Preparations showing *tufted hairs*, in which numerous unicellular filaments are inserted on a multicellular foot, may be obtained from the leaf of species of *Correa*, or from the calyx of *Lavatera* and other Malvaceæ. *Shaggy hairs*, consisting of several longitudinal rows of cells cohering laterally, are to be prepared from the leaf of *Hieracium pilosella.*

Scales have been alreadynoted in *Hippuris* (p. 110), and may also be prepared from the leaf of species of *Elæagnus*, &c.

In order to see *glandular capitate* hairs, transverse sections may be made of the petiole of *Primula sinensis:* mount in water, and observe the conical, multicellular stalk of the hair, and the round terminal head, surmounted by a highly refractive, yellowish patch. This highly refractive body consists of a resinous substance secreted below the cuticle, and the resin may be dissolved by alcohol or ether. Compare glandular hairs from the Labiatæ, &c.

A similar glandular secretion is found in connection with scales in *Humulus*, where it appears on the upper surface of the scale, and in *Rhododendron ferrugineum*, &c., where it occurs between the cells of the scale. Compare also the massive glandular *colleters* on the inner surface of the bud-scales of the Horse-Chestnut: these secrete in large quantities a clear and sticky balsam. For a description of other forms of hairs, both secretive and non-secretive, and for a more detailed account of the place and mode of secretion of resins and essential oils, reference must be made to more extended text-books.

The origin and development of *emergences* may be very well

traced in the Rose, or in species of *Rubus*, by cutting transverse sections of the young stems and petioles on which the prickles are inserted, and selecting those sections which traverse the young emergences: the sections may be cleared with "eau de javelle": it may readily be demonstrated in these that the prickles arise not from the epidermis only, but also from the subjacent tissue of the cortex.

ROOT

i. *Herbaceous Type*

Observations on the structure of the root may be made on the seedling of *Helianthus*, but as even the tap-root of this plant when young is only of small size, it will be found more convenient, and as a rule more successful, to study the structure of the root in *Phaseolus*.

Observations with the Naked Eye.

Germinate seeds of *Phaseolus multiflorus* (the Scarlet-Runner) in wet sawdust, or pure vegetable mould, till the primary root has attained a length of six to eight inches.

Note with the naked eye—

1. The **seed,** from which the testa can easily be removed, disclosing—

2. The two fleshy **cotyledons :** between these—

3. The **plumule,** which develops early as a stem, bearing foliage leaves.

4. Below the cotyledons a short **hypocotyledonary stem,** not clearly marked off externally, except by colour, from—

5. The **primary root,** on the upper part of which are—

6. Numerous secondary or **lateral roots.** These are formed in acropetal order, and are arranged in regular longitudinal rows, usually four in number. On the youngest part of the primary root (*i.e.* within three inches or more of the apex) no lateral roots are to be seen.

Observe that particles of the sawdust, &c., adhere to the older parts of the roots, while the younger apical parts come out of the soil quite clean: this is due to the fact that **root-hairs** are present on the older parts, but not on the youngest parts close to the apex.

Microscopic Observations.

Harden the roots in alcohol for two or three days or more. In order to cut the sections it will be necessary to hold the roots between pieces of pith, or, better, to embed in paraffin.

I. Cut transverse sections of the primary root at a point nearer the apex than the youngest lateral roots, *i.e.* about two inches from the end: clear the sections with weak potash, or "eau de javelle," and mount in glycerine.

Observe the following tissues:—

1. At the centre of the circular section is a mass of parenchymatous **pith.** At the periphery of this are—

2. Radiating groups of elements of the **primary xylem,** which are the most strongly marked tissues of the young root. The number of these is most often

four, but it is subject to variation, and may be as high as six: the same is the case with the groups of phloem, which alternate with these. The groups of xylem have dark lignified walls (test with chlor-zinc-iodine or aniline sulphate), and resemble the primary xylem of the stem. Note fresh elements in course of formation at their central limit: the development is thus centripetal. Alternating with these may be seen—

3. Four groups of **primary phloem,** which are not as yet very well marked. These several groups of elements are separated laterally from one another by bands of parenchyma. At the periphery of the central cylinder thus built up is—

4. The **pericycle,** consisting of thin-walled cells arranged in an undulating band, which is a single layer of cells in thickness peripherally to the phloem, but opposite the xylem it consists of two to three layers of cells.

5. Immediately outside this is the **bundle-sheath,** or **endodermis,** consisting of a single layer of cells, having the characteristic **dark dot** on their radial walls. Then follows—

6. The parenchymatous **cortex,** a thick band of tissue, with intercellular spaces, and—

7. The **piliferous layer,** a single superficial layer, not well marked. Single cells of this layer will be seen to have grown out perpendicularly to the surface as **root-hairs,** which as a rule are not branched, and are of cylindrical form, with thin cell-walls: particles of soil may be found attached to many of them, while they may often be seen to be modified in form so as to apply themselves closely to the grains of sand, &c., with which they have come in contact.

II. Cut sections successively at older points in the same root, treat as before, and observe the mode of origin of the **lateral roots,** noting more especially the following facts:—

a. The lateral roots arise opposite the groups of primary xylem: this explains their arrangement in four rows as above observed with the naked eye, since the number of groups of primary xylem is usually four.

b. The pericycle, endodermis, and a small portion of the cortex, all take part in their formation.

c. In the older lateral roots it may be seen that their vascular system is continuous with that of the main root.

d. The lateral roots, increasing in length, burst through the outer layers of cortex and the piliferous layer: since they originate from deeply seated tissues and rupture the more superficial ones, they are said to be of endogenous origin.

This mode of origin of the lateral roots is the rule in the plants with apical meristem, arranged according to Type II. (see below, p. 159). In the plants whose root-apex follows Type I. the lateral roots are mainly, or even entirely, derived from the pericyle: this is the case with *Helianthus*, and sections should be made from its roots to illustrate this point.

III. Cut transverse sections of the root, six inches or more from the apex, avoiding the lateral roots: take care also to avoid the thick base of the hypocotyledonary stem, which shows a structure characteristic neither of the stem nor of the root: treat as before.

The general arrangement of tissues is the same as has been above described, though there has been increase in bulk, and the xylem and phloem, being now more fully developed, are more easily recognized. Ob-

serve especially that the parenchyma, lying centrally to the phloem, has begun to divide repeatedly by tangential walls: in fact, four **cambium** bands are thus formed, from which is derived the **secondary thickening** of the root.

IV. Cut transverse sections of an old root of the Scarlet-Runner, taking care here also to avoid the base of the hypocotyledonary stem, and treat as before. Observe—

1. Centrally a parenchymatous **pith.**

2. The **primary xylem** groups, usually four in number, retain their original position, relatively to the pith.

3. Four large wedges of **secondary xylem** have originated internally from the four cambium zones. These are separated from one another laterally by—

4. Four broad **parenchymatous rays,** which lie on the same radii as the primary xylem. Outside the xylem is—

5. The **cambium,** having similar characters to that of the stem, and giving rise peripherally to—

6. **Secondary phloem.** Note if possible—

7. The four groups of **primary phloem** now separated from the primary xylem, but still on radii alternating with the latter. The section is bounded by—

8. A narrow band of **cork** with a **cork-cambium** at its inner limit: this originates from the pericycle, and this point should be ascertained by cutting sections successively at older points. It is to be noted that the endodermis, cortex, and piliferous layer, are absent in these sections, these being thrown off on the formation

of the layer of cork from the pericycle beneath them, which thus cuts them off from a physiological connection with the central cylinder. (Compare Fig. 10, p. 156.)

For comparison with the above, and especially with regard to the broad primary medullary rays, sections should be cut of old roots of *Cucurbita*, and of the common Nettle, both of which show parenchymatous rays of large size.

Comparisons should also be made of transverse sections of the root of the Radish, the secondary tissues of which consist chiefly of parenchyma with the vascular elements scattered through it in small groups.

ii. *Ligneous Type.*

For comparison with the root of *Phaseolus*, which is characteristic rather of that of herbaceous plants, observations should also be made on the roots of some woody plant. The root of the Elm has its cortex densely crowded with mucilage-cells, and in other respects is not very suitable for work: the Horse-Chestnut (*Æsculus Hippocastanum*) is a better type of a root of an arboreous plant. Dig up roots of this plant carefully so as not to break off the finer fibrils: wash them gently from the soil, and observe the reddish-brown colour of the thicker and more mature parts, while the ends of the thinner fibrils are pale-coloured. Note also on passing from young portions to the older that an outer coating of effete brown tissue is thrown off, and thereby the bulk of the root may be apparently diminished; this is the cortex, which here, as in other

cases, is only a temporary covering of the younger portion of the root. The soil will be found in this case also adherent to the fibrils, thus indicating the presence of root-hairs at some distance from the apex, but not at the extreme apex, nor on the older portion of the root where the cortex has been thrown off.

V. Select a strong young fibril: embed a short piece of it, taken about half-way between the apex and the beginning of the red colouring: cut transverse sections, and clear with potash, or "eau de javelle": mount in glycerine, and examine under a high power: observe—

1. The **piliferous layer,** a rather irregular layer with slightly thickened outer wall: single cells may have developed as **root-hairs.**

2. The **exodermis** showing a thickening of the radial walls not unlike that common in endodermis: this thickening is continued all round the anticlinal walls, and is not uncommon in roots of woody plants.

3. A broad band of ordinary **cortex,** with large intercellular spaces: the walls are more thickened nearer the central cylinder, and are pitted.

4. The **endodermis** or bundle-sheath, with the characteristic dot on the radial walls: within this is the central cylinder, composed of—

5. A peripheral layer of the pericycle: occasional periclinal divisions may be seen in the cells of this layer.

6. The groups of **primary xylem**—usually *four* in number—which will be still separate from one another, or, according to the age of the root, may be united at the centre: note the details of appearance of this primary

xylem, so as to be able to recognize it in the older roots.

7. The groups of **primary phloem,** equalling in number those of the xylem: this tissue is not well marked.

8. Narrow bands of parenchyma separating the successive groups of phloem and xylem: subsequently these cells are the seat of origin of the cambium.

VI. Cut similar transverse sections from a part of a root which has recently turned brown, and has produced lateral roots: comparing with the above sections, note the following changes:—

1. The piliferous layer and cortex have turned brown, and will often be separated from the central cylinder by rupture of the radial walls of the endodermis.

2. The cells of the pericycle will be found undergoing periclinal divisions, which ultimately result in the formation of a layer of cork.

3. The groups of primary xylem as before, but united at the centre so as to form a star-shaped mass, with *no central pith:* the groups of primary phloem alternating with those of primary xylem, as before.

4. Divisions may be seen in the cells intervening between the xylem and phloem, which indicate the position of the **cambium.**

5. If lateral roots be present, they originate from the pericycle.

VII. Cut transverse sections of still older portions of the root, about $\frac{1}{16}$ of an inch or more in diameter treat with chlor-zinc-iodine, which brings the starchy medullary rays into prominence. Now examine carefully the central mass of tissue, and recognize the

primary xylem in its original position: the further arrangement of the secondary tissues is as in the Scarlet-Runner, but note these points of difference in the root as a whole :—

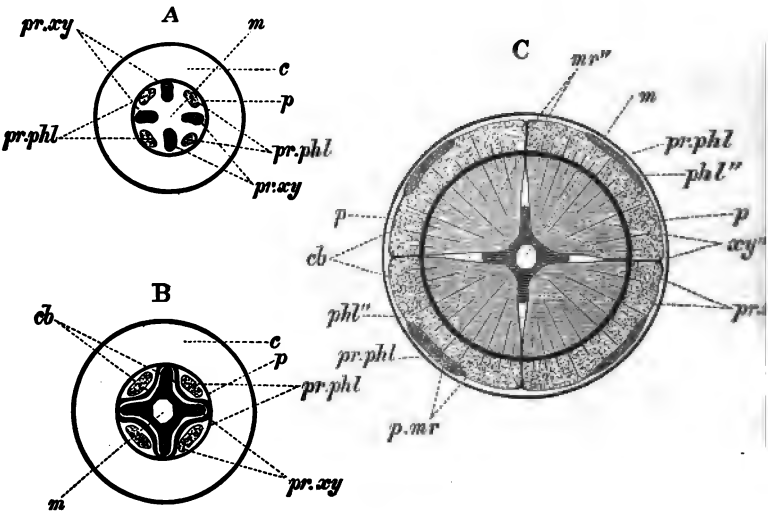

FIG. 10.—A. Diagram illustrating the disposition of tissues in the young root of a Dicotyledon, before the cambial divisions begin.

B. The same at a later stage, when the cambium may be clearly recognized.

C. Diagram of arrangement of tissues in the root, after secondary thickening has been in progress for a considerable time : in such a root the cortex, which is seen as a broad band (*c*) in Figs. A, B, has been completely thrown off, and the section is now limited by tissue (*p*) derived from the deeper-lying pericycle.

c = cortex ; *p* = pericycle ; *pr.xy* = protoxylem ; *pr.phl* = protophloem ; *m* = pith ; *cb* = cambium ; *xy''* = secondary xylem ; *phl''* = secondary phloem ; *p.mr* = primary medullary rays ; *mr''* = secondary medullary rays.

1. There is no pith.

2. The primary medullary rays opposite the groups of primary xylem are relatively small and inconspicuous.

3. The xylem is marked off into annual rings, as in the secondary xylem of the stem.

It is further to be observed that the cortex has completely disappeared, and the peripheral layer of cork is the product of the pericycle. Numerous sclerenchyma-fibres are to be found scattered through the secondary phloem.

The diagrammatic Fig. 10 will illustrate the mutual arrangement of the primary and secondary tissues of the root.

Apex of the Root.

Type I.—Using the fruit of the Sunflower in the dry state, as it may be bought in seedsmens' shops, cut thin median longitudinal sections of the apex of the radicle of the straight embryo. The arrangement of the meristem at the apex of the radicle of the embryo is similar to that of the apex of the growing root, and the former is chosen in this case as it is much easier to make preparations from it than from the growing root. The sections are of little use unless they are accurately median.

Treat the sections with potash for ten minutes or more, or, better, treat with "eau de javelle" as directed on p. 50: wash with water, and mount in glycerine: examine with a low power, and observe that—

1. The mass of tissue is composed of thin-walled cells, arranged regularly in longitudinal rows.

2. That these rows of cells converge towards a point at some distance below the external apex of the root. This is the *punctum vegetationis.*

3. Note the **procambium-cylinder,** or formative tissue of the vascular bundles, which pursues a longitudinal course up the centre of the root.

The general scheme of arrangement of the apical meristem is indicated in the diagram Fig. 11 : but in comparing the sections with the diagram it must be remembered that the figure represents an ideal Dicotyledonous type, and it must not be attempted to trace a correspondence of minute detail of the sections with

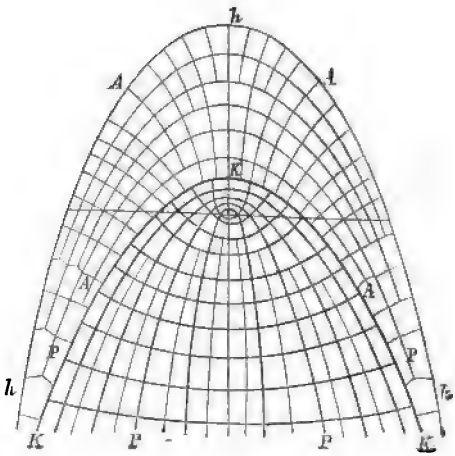

FIG. 11.—Diagram illustrating the arrangement of meristem which may be found at the apex of a root of a Dicotyledon. h h h, the root-cap, limited internally by the line K K K, which represents the outer limiting wall of the dermatogen. In the root-cap the letters A A indicate anticlinals ; P P, the periclinal curves. (After Sachs.)

the diagram: thus in the diagram there is a sharp limit K K, between the root-cap and the body of the root, whereas in the Sunflower, as will be presently shown, the root-cap and piliferous layer have a common origin.

Examine with a high power: and observe that—

1. At some distance from the apex a definite

piliferous layer covers the root externally. Follow this towards the apex: at some short distance from it this single layer splits into two: the inner is the **dermatogen,** formative of the piliferous layer: the outer is the outermost layer of the **calyptra,** or root-cap. Following the dermatogen further inwards, it will be seen to split again several times in succession: the dermatogen may be traced as a continuous layer covering the inner tissues. The layers thus thrown off externally from the dermatogen form collectively the **root-cap,** or **calyptra.** We have in this case a common formative layer for root-cap and piliferous layer.

2. Between the procambium and the piliferous layer lies abroad band of formative tissue of the cortex, or **periblem:** follow this to the *punctum vegetationis:* it is also a distinct continuous band, though reduced to a single layer of cells at the apex.

3. The **plerome,** or central procambium cylinder, may also be traced as distinct up to the apical point.

This type of arrangement of tissues of the meristem may then be expressed thus:

Calyptrogen } a single layer of cells, *i.e.* piliferous layer
Dermatogen } and root-cap have a common origin.
Periblem, distinct from the rest.
Plerome, distinct.

To this type belong most of the Dicotyledons. The work may be equally well done on *Linum usitatissimum* or *Polygonum Fagopyrum.*

Type II.—Prepare median longitudinal sections of the apex of the radicle of *Phaseolus multiflorus* (the Scarlet-Runner), or perhaps better, from the Broad-Bean:

in either case the dry seed may be used: treat as the above. Examine with a low power, and make out—

1. Calyptra (root-cap).
2. Piliferous layer.
3. Periblem.
4. Plerome, forming the procambium and pith.

But here all the different tissue-systems will be found to originate from a general meristem, the original formative tissue of none of them being distinct from that of the others. This type may be expressed, shortly, thus:—

Calyptrogen Dermatogen Periblem Plerome	All united in a general, undifferentiated mass of meristem.

As alternative plants of the same type, may be named *Cucurbita* and *Pisum*.

VEGETATIVE ORGANS.—(B) MONOCOTYLEDONS

i. STEM—*HERBACEOUS TYPE*

I. Cut transverse sections from about the middle of an internode of a well-grown stem of *Zea Mais:* mount in water, or glycerine.

N.B.—Fresh material may be used, but stems preserved in alcohol are preferable, for when fresh the tissues are crowded with air-bubbles. The sections should be cut from the upper part of one of the lower internodes, otherwise the vascular bundles may be found to be imperfectly developed.

Examine with a low power, and, beginning the study of the tissues at the periphery of the section, observe—

a. A single layer of rather irregular **epidermis:** immediately below this are—

b. Irregular groups of **sclerenchyma** with thick lignified walls: internally lies—

c. A mass of **parenchyma,** which forms the groundwork of the whole section: embedded in this are—

d. Numerous **vascular bundles:** note that they are smaller, but more numerous near the periphery

than at the centre; also that the position of the parts of the bundles relatively to the centre of the section is usually uniform.

Treat a section with chlor-zinc-iodine: put on a high power, and examine in detail the several tissues above named.

a. The **epidermis** appears as a definite layer of cells of unequal size, without intercellular spaces. Note a well-marked **cuticle** (brown). Here and there may be found **stomata,** with two small **guard-cells,** and two **subsidiary cells**: the structure and development of the stomata will be studied in the leaf; p. 175.

b. The **sclerenchyma** consists of cells with thick, highly refractive walls, which stain yellowish brown with chlor-zinc-iodine (lignified). Note that it does not occur immediately below the stomata, but, as usual, there is there an intercellular space (air chamber).

c. The **parenchyma** consists of cells with thin cellulose walls (blue with chlor-zinc-iodine). At the angles where the cell-walls meet are intercellular spaces. The external layers have abundant protoplasm with chlorophyll-corpuscles. These are less frequent in the inner layers, while in the central parenchyma the protoplasm is hardly appreciable.

d. For the minute study of the **vascular bundles** select one of the largest central bundles. The section must be thin. The most prominent elements in the bundle are—

i. Four large **vessels** of the **xylem,** arranged like a **V,** with the angle towards the centre of the stem:

of these the two smaller are developed first. Compare sections of young stems.

In many Monocotyledons the arrangement of the constituents of the xylem in the form of a **V** is much more plain than here, *e.g. Asparagus.* In other cases (*e.g. Calamus*) this arrangement is not to be seen.

The vessel nearest the centre of the stem has usually **annular** thickening: in old stems it is partially surrounded by an intercellular space, while the rings often become detached, in which case the vessel is not easily seen in transverse sections. Next this is a vessel which has commonly a **spiral** thickening: the remaining two have thinner walls with **pitted** marking, and large cavity.

Surrounding the pitted vessels, and between them, are—

ii. A number of **tracheides** with pitted lignified walls, and no cell-contents. Surrounding the intercellular space above described is—

iii. A group of parenchymatous cells with thin cellulose walls. These may be regarded as **xylem parenchyma.**

The **phloem** portion of the bundle lies between the limbs of the **V**-shaped xylem, and is easily recognized by the thin cellulose walls characteristic of **soft bast.** It consists of—

iv. Elements with large cavities, in which transverse septa (sieve-plates) often occur: these elements are the **sieve-tubes.**

v. Smaller cells (**cambiform**) between the sieve-tubes.

2. The entry, horizontal course, and fusions of the bundle-system of the leaf, and axillary bud.

Note that the plexus of bundles at the node does not extend far in a perpendicular direction.

V. In order to see the fundamental arrangement of the vascular system, cut median longitudinal sections through the apex of a young plant of Maize, or of a foliage-branch of an old plant: treat with strong potash; or, better, with dilute potash for twenty-four hours: examine with a low power, and observe, if the section be median—

1. The **apical cone** (*punctum vegetationis*).

2. **Leaves,** in successive stages of development, seated laterally.

3. In the older leaves, **vascular bundles,** which enter the stem.

On following the course of these vascular bundles it will be seen that on entering the stem they proceed at first towards the centre: before reaching it they curve downwards, and finally turning again outwards they approach the periphery of the stem. We thus see that in young stems of Maize the course of the bundles corresponds to the Palm-type, though as the stem grows older, and the internodes develop, the correspondence is less obvious, by reason of the almost straight course pursued by the bundles in the internode, and the complications which arise at the node.

But no student should be satisfied with seeing the typical bundle-system of a Monocotyledon in small microscopic preparations: for it is not difficult to prepare from any of the Palms which have a columnar stem, dissections which shall show plainly to the naked

eye the course of the vascular bundles. The spiral lateral curvature of the bundles in their downward course may be readily recognized in such dissections, where the ground tissue has been removed to a sufficient depth. No botanic institution should be without such dissections, which will make more plain to the mind than any description, or any microscopic preparation, the rather complicated bundle-system of the Palm-type.

ii. STEM—*BULBOUS TYPE.*

I. Examine a plant of one of the cultivated varieties of *Hyacinthus orientalis* in flower, and note—

1. The broadly conical **bulb** covered externally with dry scales, and having at the base a large more or less concave scar covered with corky tissue.

2. A fringe of unbranched **roots** rising from the periphery of the scar.

3. The **leaves,** which are of two sorts, (*a*) **scale-leaves,** which are colourless, short, and broad, and constitute the greater part of the bulb, and (*b*) long, narrow, and green **foliage-leaves,** with parallel venation.

Halve the bulb longitudinally, and recognize the shortly conical, yellow **axis** at the base of the bulb, which is of small bulk compared with the bulb as a whole, and is elongated upwards as the cylindrical peduncle or **scape.** Upon this short axis are inserted the fleshy, sheathing scale-leaves, and the less fleshy bases of the foliage-leaves : note that in the latter there is no clearly marked distinction between sheath and lamina, as in the Maize.

Here and there buds may be seen in the axils of the scale-leaves, which repeat the characters of the main bulb.

The external details of the inflorescence may be deferred for the present.

II. Cut transverse sections of the scape: mount in glycerine, and examine under a low power: observe that the general arrangement is not unlike that in *Zea*.

1. There is a superficial **epidermis,** of thick-walled cells.

2. A general **ground-tissue,** without sclerenchyma, in which are embedded—

3. The **vascular bundles**: these range themselves as a more or less regular external series of smaller bundles, and an internal series of larger bundles.

Treat some sections with chlor-zinc-iodine, and examine under a high power: observe—

1. That the **epidermis** is a regular layer of cells, with thickened and stratified outer and inner walls consisting of cellulose (blue), clearly marked **cuticle,** which is here found limiting the surface of both the outer and inner walls: note **stomata** with two large guard-cells.

2. The **ground-tissue** consists of cells having thin cellulose walls, and containing chlorophyll: it is divided into an external cortex, and an internal mass, by an irregular band of cells with a slight collenchymatous thickening: this band runs externally to the outer series of bundles.

3. The **vascular bundles** are of the collateral type, having the xylem directed to the centre of the stem, and maintaining, though not very clearly, the form of the

letter V : it is more bulky than, but not so regular as, in the Maize. The phloem lies between the limbs of the V. The bundles are not sharply defined from the ground tissue : very small bundles are scattered irregularly towards the centre. Here and there may be found cells with raphides.

Longitudinal sections should also be cut, and a comparison made with the appearance of the tissues as seen in the transverse sections.

iii. STEM—*ARBOREOUS TYPE.*

I. Examine preparations of the old stem of *Yucca* or *Dracæna*, in which the thin-walled parenchyma has been allowed to rot away, while the vascular bundles remain. On comparing transverse and longitudinal sections of such stems, it may be seen, with the naked eye—

1. That the central **primary bundles** are isolated, and that the course of each bundle may be traced as starting from below at the periphery of the stem, then curving towards the centre as it ascends, and finally turning outwards, and passing into a leaf. These are therefore **common bundles.**

2. That the peripheral mass of secondary bundles increases in thickness towards the base of the stem, and has no direct connection with the leaves. These bundles are therefore **cauline.**

II. Cut transverse sections of the stem of *Dracæna* immediately below the apical tuft of leaves : mount in glycerine, and examine under a low power. Observe

that the arrangement of the tissues, as well as the character of the vascular bundles, is similar to that in the Maize : there is, however, a peripheral cortex, which is not found in the Maize. Note that the cells of the inner limit of the cortex are quiescent, and not undergoing division.

III. Cut transverse sections of the stem of *Dracæna* at a point one foot or more from the apex, and mount in glycerine. Examine with a low power, and observe—

1. A well-marked **epidermis.** Beneath this—

2. A band of **cork.**

3. A broad belt of **cortical parenchyma,** many cells of which contain crystals (raphides, &c.). Here and there a vascular bundle will be seen in the cortex : these are bundles of the leaf-trace, passing inwards from the leaves.

4. At the inner limit of this the cells are not quiescent, as in the younger part of the stem, but there is an actively dividing **meristematic ring,** which gives rise internally to new vascular bundles, and externally to fresh cortical cells. The new bundles thus formed are **cauline,** having no direct connection with the leaves, and are embedded in lignified ground-tissue. These together form a dense ring.

5. Centrally, there still remains undisturbed that arrangement of thin-walled **parenchyma** and **vascular bundles** which has been above noted in the young stem as being similar to that in the internode of Maize : the primary or common bundles may be distinguished from the secondary or cauline bundles, not only by their arrangement, but also by their structure, the latter

having a denser sclerenchymatous sheath and smaller vascular elements than the former.

Note the passage of these central bundles outwards to the bases of the leaves: they are **common** bundles. Note also the mode of formation of the cauline bundles.

iv. STEM—*AQUATIC TYPE.*

I. The American Water Weed (*Elodea canadensis*) may be taken as an aquatic type: it grows habitually submerged, with its leaves arranged in whorls, and with elongated internodes.

Cut transverse sections of an internode: mount some in water or weak glycerine, others in chlor-zinc-iodine: note in the circular transverse sections—

1. The regular epidermis, with no stomata.

2. The broad band of cortex, with intercellular spaces of various size: six small strands of prismatic cells may be recognized.

3. The bundle-sheath, consisting of relatively small cells, having the usual dark dot on the radial walls: this will be best seen after treatment with potash.

4. The central vascular strand, consisting of thin, walled tissue with **no lignified walls whatever:** centrally is a round cavity (vessel), but without lignification of the walls.

Chlorophyll-granules and starch may be found throughout the tissues. This is evidently a more thoroughly aquatic type than *Hippuris*, and its completely submerged habit is to be remembered in connection with the simpler internal structure.

II. Longitudinal sections should also be cut, when it will be found that the central cylinder consists chiefly of parenchyma, with a few sieve-tubes: note also the central vessel.

The radial walls of the bundle-sheath may often be well seen, especially in slightly oblique sections: note the wavy outline of the walls, to which the appearance of a dark dot in the transverse section is due. (Compare p. 76.)

i. Leaf—*BIFACIAL TYPE.*

Note that the phyllotaxis in the Maize is $\frac{1}{2}$: the leaf is sessile, and sheathing in its lower half, with a ligule at the upper limit of the sheath; the form of the lamina is lanceolate, margin entire, ciliate, midrib well marked; venation parallel; upper surface hirsute; lower glabrous.

I. Treat a piece of the thin peripheral part of a leaf (which has been previously bleached in alcohol) with potash till it is transparent; mount in glycerine, and examine under a low power. Observe—

1. The **parallel** course of the **vascular bundles.**

2. Their frequent **lateral fusion,** by means of small branch-bundles.

3. The absence of **stomata** above the vascular bundles, and their arrangement in rows in the spaces between them.

4. The various forms of **hair;** and especially the conical unicellular hairs, which give the ciliate character to the margin of the leaf.

II. Cut transverse sections of the lamina; mount in water, or dilute glycerine.

Other sections may be treated with alcohol to expel the air-bubbles; the chlorophyll will, at the same time, be dissolved out: the sections may be mounted in chlor-zinc-iodine, and kept for comparison with the above.

Examine with a low power.

The section presents a sinuous outline, corresponding to a certain extent to the arrangement of the main vascular bundles: at the midrib the section widens out. Note the following arrangement of tissues:—

1. Covering both surfaces of the leaf is an **epidermis,** resembling that of the stem, but bearing **hairs** of various form, mostly simple, conical: the largest of them are surrounded at the base by an outgrowth of the neighbouring epidermal cells.

Note the **stomata** on both surfaces, with small guard-cells, surrounded by two subsidiary cells: these will be further examined below.

2. **Vascular bundles** of various size, which, in the thinner part of the lamina, lie in a median position between the two epidermal layers. The largest of these correspond in structure to those of the internode, the smaller ones are reduced forms of the same type. Note that the spiral and annular vessels (*i.e.* protoxylem) are nearer the upper surface of the leaf.

Between the epidermis on either side, and the larger bundles, are masses of **sclerenchyma,** which, together with the bundles, form complete bridges of rigid tissue

between the two epidermal layers. Compare the phyllodes of Acacia (pp. 135).

3. The spaces between the tissues hitherto considered are filled with **parenchyma (mesophyll)**, which may either be (*a*) green (containing chlorophyll), or (*b*) colourless (without chlorophyll).

a. The green chlorophyll-containing parenchyma fills up the greater part of the space; intercellular spaces occur in it.

b. The colourless parenchyma occurs (i.) as a sheath, without intercellular spaces, surrounding each bundle (bundle-sheath); (ii.) as groups of cells immediately below the epidermis: these are more common towards the central part of the leaf. At the midrib this tissue forms the bulk of the structure.

III. Cut transverse sections of the leaf-sheath, and treat as the above. Compare the arrangement of tissues with that of the lamina, and of the stem. Note that colourless parenchyma preponderates.

IV. Cut thin tangential sections from the under surface of the lamina, so as to remove, if possible, only the epidermis. Treat with potash, and mount in glycerine. Observe—

1. The ordinary cells of the **epidermis,** of oblong form, and with sinuous outline.

2. Short cells between the ends of these, which often project perpendicularly to the surface as **hairs** of various form.

3. The **stomata,** holding the same position as (2) relatively to the oblong epidermal cells.

Observe with a high power the structure of the stomata. They consist of—

a. Two narrow **guard-cells,** which inclose the **pore.**

b. Two triangular **subsidiary cells,** which completely surround the convex side of the guard-cells.

Compare this view of the stoma with the same structure as seen in transverse sections of the lamina.

V. Cut tangential sections of the upper surface of the lamina. (1) Mount some, and examine them under a low power. (2) Treat others with nitric acid; dry them, and ignite on platinum foil over a spirit-lamp. Mount the ash in water, and examine under a low power. The structure will resemble that of (1).

Treat with acetic acid: no evolution of gas.

Treat with nitric acid: it is not dissolved.

The residue is a **silica-skeleton** of the epidermal tissues. (Compare p. 90.)

VI. Transverse sections should also be cut from the foliage-leaves of the Hyacinth, which, as above noted, grow in an almost vertical position: mount in glycerine, and note under a low power that there is no great difference between the upper and lower surfaces as regards the disposition of the tissues, excepting that the orientation of the collateral bundles is such that the xylem is directed towards the upper surface.

Stomata may be seen both on the lower and upper surfaces: there is no distinctly marked palisade-parenchyma: centrally is a mass of colourless thin-walled parenchyma. Note the absence of strengthening sclerenchyma.

This will be found an excellent opportunity for the study of the **details of a simple stoma:** observe that the guard-cells are about at the general level of the

epidermis: that when fairly cut through the middle they differ in section from the other epidermal cells: the cell-wall separating them from the adjoining epidermal cells is relatively thin, while that adjoining the pore of the stoma is thick, excepting at one point, where the guard-cells are near to, or in contact with, one another. There are external and internal thickened ridges on each guard-cell, which appear in section as sharp projecting teeth: these respectively define and partially inclose the front cavity and back cavity of the pore. The cuticle is seen to be continuous through the pore, to the lower surface of the epidermis. Note also the large air-chamber leading into the system of intercellular spaces of the cortex. The contents of the guard-cells are conspicuously stained blue with chlor-zinc-iodine.

The above are the main characteristics of the guard-cells of most stomata, but they can seldom be so readily observed as in this case.

VII. To observe the stoma in surface view, and in the living condition, take leaves of Hyacinth, or better of some species of *Lilium*, in which the stomata are of unusually large size, on a bright day, and after full exposure to the light, strip off a piece of the epidermis, and mount it in water, with the outer surface uppermost, and examine under a low power. It may then be readily seen that the **pores** of the stomata are **widely open,** the guard-cells being strongly curved.

Having seen this, irrigate with a 2 per cent. solution of common salt, keeping watch upon a stoma which has been seen to be open: when the salt solution reaches it, the stoma will be seen to close, the guard-

cells straightening themselves as their internal tension is relieved, and finally becoming plasmolysed. The connection between the opening of the stoma and the internal tension of the guard-cells is thus demonstrated.

It will not be amiss at this point to study the **development of the stoma.**

Take a young leaf or young scape from a bulb of *Hyacinthus orientalis* in which the leaves have not yet protruded more than about one inch from the apex of the bulb. Strip off pieces of the epidermis (or cut tangential sections at successive points) starting from the apex, and proceeding to the very base. Mount in glycerine, and examine under a high power.

i. Starting at the parts taken from points nearest the base, cell-division will be found to be proceeding actively in the epidermal tissue; the walls are thin, and protoplasm copious. The epidermis consists of—

a. Larger oblong cells.

b. Short, nearly square cells.

The cells are arranged in regular longitudinal rows.

ii. At a short distance from the base, the difference in size of (*a*) and (*b*) increases; some of the square cells may be seen to be divided by a thin longitudinal wall into two equal halves **(guard-cells of the stoma).**

iii. Further up again, this division wall may be seen to be thicker at its central part, while the whole outline of the pair of guard-cells tends to become circular.

iv. Again further up, the division wall will be seen to have split, so that a channel is formed between the guard-cells into the internal tissues of the leaf. This channel is the **pore** of the stoma.

v. Near the apex of the leaf the mature stomata may be seen of circular outline; their guard-cells are sausage-shaped, and surround the nearly circular pore. The cells of the epidermis remain oblong as before.

If the sections be carefully stained with Kleinenberg's

hæmatoxylin, and mounted in Canada balsam, stages of division of the nucleus may be observed in the cells which are about to divide into the two guard-cells.

It is more difficult to trace the development of the stoma of the Maize; but it may be done in the same way in a foliage-bud. The main point of difference is that after the mother-cell of the stoma has divided to form the two guard-cells, two other cells are cut off from the neighbouring epidermal cells (**subsidiary cells**): these lie parallel to the guard-cells.

Further, the epidermis of the Maize is complicated by short cells, which appear in irregular groups among the ordinary epidermal cells: this is a common character among the Grasses.

VIII. Sections should also be made through the fleshy scales of the bulb, and these will show the same general arrangement of tissues as the foliage-leaves, but the colourless parenchyma is much more bulky, and is densely stored with starch: numerous cells containing **raphides** will be found, especially towards the outer (lower) surface of the scales.

Note on crystals.

Cut longitudinal sections of the scape or leaves of the Hyacinth or Onion: many other Monocotyledons will do as well: mount in water, and observe the large cells containing numerous needle-shaped crystals (**Raphides**) arranged in a bundle parallel to one another. In order to investigate their nature the following tests may be applied:—

a. The attempt may be made to stain them with iodine, or other stains which colour crystalloids, but they will not be affected: they are thus distinguished at once from crystalloids.

b. Irrigate a section with acetic acid: they are not affected: they are therefore not calcium carbonate.

c. Irrigate with dilute nitric acid: the crystals are dissolved.

d. Irrigate a fresh preparation with a small quantity of dilute sulphuric acid: the crystals will be dissolved, and crystals of a

different form (calcium sulphate, which is not readily soluble) may be seen to be formed in the fluid.

These reactions, coupled with what can be ascertained from analysis of the ash of the plant, point to the conclusion that these crystals consist of **calcium oxalate.**

Cut transverse sections of the petiole of some species of *Begonia;* mount in water, and examine under a low power. Here and there will be found bodies of a more or less distinctly crystalline form occupying the cavities of certain cells. Their form is very complicated, and their size variable.

The reagents above applied to the Hyacinth are to be used: the results will be similar. Thus they also may be shown to consist of calcium oxalate. Crystals giving the above reactions will be found in the tissues of most plants.

ii. LEAF—*ISOBILATERAL TYPE.*

Examine the leaves of the common Iris (I. *Pseud-acorus*), and note the lower sheathing or equitant part, and the upper, laterally copmressed, ensiform portion. Cut transverse sections of the latter, mount in glycerine, and observe the general disposition of the tissues, as follows:—

1. On either surface a layer of **epidermis with stomata.**

2. Beneath each epidermis a band of **chlorophyll-containing parenchyma,** which gradually merges into—

3. A central mass of **colourless parenchyma.**

4. Distributed somewhat irregularly are numerous **vascular bundles** of various size; sometimes they are arranged opposite one another, sometimes alternately. Note especially that their orientation is such that the phloem (usually bounded by a band of sclerenchyma) is

directed towards the nearest external surface, the xylem towards the centre.

Compare the structure of the phyllode of Acacia (p. 134).

iii. Leaf—*AQUATIC TYPE.*

Cut transverse sections of the leaf of *Elodea:* fresh material may be used, and the sections may be mounted in water. On examination the flattened lateral portion of the leaf will be found to consist of two layers of cells, with intercellular spaces, but no stomata. The upper layer consists of larger, the lower of smaller cells, and both contain chlorophyll.

Note the thicker midrib, and **reduced vascular bundle,** which consists of two or three fibres of bast towards the lower surface, while towards the upper surface a small-celled tissue is all that represents the xylem.

Root.

I. Cut transverse sections of the root of the Maize, selecting a well-grown, underground piece of root: mount in glycerine, and examine first under a low power, then under a high power. Starting from the periphery observe—

1. The superficial **piliferous layer,** consisting of small cells, many of which have grown out into long **root-hairs.**

2. Immediately below this is a protective layer of **exodermis,** with more or less cuticularized cell-walls.

3. The **cortical parenchyma,** a broad band, of

which the peripheral part is often sclerenchymatous, and this is especially the case in the aërial prop-roots: observe the regularity of arrangement of the inner part of the cortex, of which the innermost layer is to be recognized as—

4. The **endodermis**: the cell-walls of this layer are in old roots thickened on three sides, the outer wall remaining thin, and the radial walls in young roots show the usual dark dotted appearance.

5. Within this is the **pericycle,** a layer of cells with walls thin when young, but they may be lignified when old: the series is interrupted opposite the xylem masses, which abut directly on the endodermis.

6. The **vascular cylinder,** round the periphery of which are disposed—

A. **Xylem-tissues,** recognized by their dark-looking lignified walls, especially those of the large vessels: these lie nearer the centre, while the smaller elements of the **protoxylem** are at the extreme periphery.

B. **Phloem-tissues,** which alternate with the xylem-groups, and are to be recognized by their brighter cellulose walls. Note that the number of xylem and phloem groups may vary, and is often very large.

7. Centrally lies a bulky **pith,** in which may be seen one or more irregular groups of sclerenchyma surrounding a vessel or vessels.

II. Cut transverse sections of the root of *Hyacinthus orientalis.* An old root must be taken, and the sections should be cut as far as possible from the apex. Treat them with potash, and mount in glycerine. Starting from the outside, note successively—

1. The superficial **piliferous layer,** single cells of

which have grown out, here and there, perpendicular to the surface as **root-hairs.**

2. Beneath this a layer of cells of larger size with clearly-marked, cuticularized cell-walls: this is the **exodermis.**

3. A thick band of **cortical parenchyma,** consisting of rounded cells with intercellular spaces: in old roots the outer layers of this tissue become disorganized and distorted. The inmost layer of this tissue differs in structure from the rest, and is called—

4. The **endodermis:** the radial walls of this layer present the characteristic appearance of a black dot, and are cuticularized.

5. Within this is a layer of thin-walled cells of the **pericycle** which immediately surrounds—

6. The central **vascular cylinder:** two kinds of tissue are specially to be distinguished in this cylinder, viz.,—

A. **Xylem-tissues,** easily recognized by their dark lignified walls: they are arranged in a series of groups of indefinite number, which abut externally on the pericycle, and extend inwards, till they meet internally, and form a central mass. The chief constituents are vessels of various form. As may be seen in transverse sections of young roots, the smaller peripheral members of each group are formed first (**protoxylem**), and have spiral thickening; then successively the larger vessels towards the centre.

B. The **phloem-tissues,** which are groups of elements with small cavity, and bright cellulose walls: they lie between the peripheral groups of the xylem, and alternate regularly with them.

III. Cut radial longitudinal sections of the same root: treat in the same way, and observe the several tissues above described. The whole root will be seen to be composed of similar elements to those found in the stem. Note especially that the spirally thickened elements of the xylem (protoxylem) are next to the pericycle.

In the transverse sections may be found the point of junction of lateral roots with the main root. It may be seen that the former originate from the pericycle of the main root, and that they break through the endodermis, cortical tissue, and piliferous layer; also that their vascular tissue is continuous with that of the main root: the activity which produces them begins opposite a phloem-mass of the main root, and not opposite a xylem-mass, as is usually the case. This is to be connected with the fact that the xylem groups in the Maize (and in most Grasses) abut directly on the endodermis.

Apex of the Root.

It is not easy to cut longitudinal sections of the apex of an ordinary fully developed root without embedding The arrangement of the meristematic tissues is, however, the same in young as in old roots; it is therefore more convenient, and quite as successful, to cut longitudinal sections of the apex of the young lateral roots, which are to be found growing horizontally out of the nodes of the Maize plant. Or, if fitting material for this be not at hand, longitudinal sections may be made of the radicle of the embryo.

Adopting one of the above methods, cut longitudinal median sections of the apex of the root. The section

must be accurately longitudinal and median, *i.e.* the section must include the organic axis of the root, around which the several tissues are symmetrically arranged.

Treat the sections for about ten minutes with dilute potash, or, better, with "eau de javelle" (Appendix A), and mount in glycerine.

In a median section the following arrangement of tissues will be visible :–

1. A central mass of tissue, clearly defined laterally, and rounded off at its apex, which is at some distance below the external apex of the root: this is the **plerome-cylinder.** If this tissue be traced back into the older part of the root, it will be found that its central part is continuous with the parenchymatous pith, while its peripheral part develops into the vascular ring. Note rows of larger cells, which may be traced back as continuous with the vessels of the xylem. In the central portion of the plerome are intercellular spaces, which appear black in sections from fresh material, being filled with air.

2. Surrounding the plerome is a broad band of tissue with intercellular spaces, which appear as irregular black lines. This tissue is the **periblem,** which is the formative tissue of the cortex.

3. Outside this is a single layer of cells somewhat elongated radially, and with a thick outer wall: this is the **dermatogen,** or formative tissue of the piliferous layer.

If the section be accurately median it will be possible to trace (2) and (3) upwards, till, immediately above the apex of the plerome, they merge into a single layer of cells: thus the formative tissue from which the

piliferous layer and cortex are derived is represented at the apex by a single initial layer of cells.

4. Outside the dermatogen, at the apex of the root, will be found another formative tissue, the cells of which divide parallel to the surface of the dermatogen: this is the **calyptrogen**-layer, which is formative of the tissues of the **root-cap.** The latter appears as a mass of parenchyma, covering the whole apex of the root: the outer cells of it will be seen to be undergoing disorganization, and mucilaginous degeneration of the cell-walls.

Aërial Roots.

Examine fresh aërial roots of *Vanda*, *Oncidium*, or some other Orchid, and note the greyish appearance of the outer band of tissue or **velamen**, and the green colour of the tissues beneath: also the smooth green apex of the root.

Cut transverse sections from the mature part of the root of *Vanda*: other epiphytic Orchids will do as alternatives, though differing in minor details: mount in glycerine, and observe—

1. A peripheral band of tissue about five layers of cells thick, which constitute the **velamen**: note the fibrous thickening of the walls, and absence of contents in this covering of extra-fascicular tracheides.

2. At the inner limit of the velamen is a definite layer of the **exodermis**, a protective tissue which is not uncommonly found beneath the piliferous layer of roots which retain their cortex.

3. Within this a broad band of **cortex**, containing chlorophyll, and many cells contain raphides.

4. Centrally lies the **vascular cylinder**, having an interruptedly thickened **endodermis**, and alternating but ill-defined groups of xylem and phloem: a few isolated groups of vessels and sclerenchyma may be found scattered about the centre of the cylinder, and this is not uncommon in large roots of Monocotyle-

dons: it is especially well seen in the aërial roots of *Pandanus* or in the larger roots of *Dracæna*.

Cut tangential sections through the velamen, and observe the irregular form of the tracheides, and the uneven thickening of the walls, the extreme outer wall being beautifully marked: where the sections have traversed the exodermis note the wavy appearance of the radial walls (see p. 76), and the somewhat regular alternation of long and short cells: the walls of the longer cells are distinctly cuticularized.

Longitudinal median sections should be prepared through the apex of the root: in these the **root-cap** will be seen covering the tip, externally to the velamen.

REPRODUCTIVE ORGANS

Observations with the Naked Eye on the Mature Flower

In order to become acquainted with the external characters of the reproductive organs, it will be well to examine and compare a few common types of flower; and since the sexual reproductive process is essentially the same in Dicotyledons and Monocotyledons, the two may be treated simultaneously.

I. Examine specimens of the common Buttercup (*Ranunculus acris*): a number of **flowers** may be found associated together on a single branching system, the **inflorescence,** which has here the character of a cymose panicle. Recognize in each single flower the following series of parts, which are inserted upon the enlarged apex of the axis or **floral receptacle**:—

1. The **calyx,** which is the outermost series of floral leaves, and consists of five sepals, separate from one another (or polysepalous), inserted below the other organs (inferior), greenish yellow, and hairy.

2. The **corolla,** consisting usually of five yellow **petals,** separate from one another (polypetalous), and seated below the more central organs (hypogynous): remove a single petal, and observe the pocket-like **gland** or **nectary** on the upper surface, close to the base.

3. The **androecium**, consisting of an indefinite number of **stamens**, which are separate from one another (polyandrous), and are seated below the central series of organs (hypogynous): each stamen is a club-shaped body, and two parts of it are to be recognized—

a. A thin stalk, the **filament.**

b. A two-lobed head, the **anther.**

In a flower fully opened, note with a lens the dehiscence of the anthers by two longitudinal slits, through which the powdery yellow **pollen** may escape.

4. The **gynoecium** at the centre of the flower, consisting of an indefinite number of **carpels**, which are separate from one another (apocarpous), and are seated above the other floral organs on the conical receptacle (superior). Each carpel consists of a lower laterally compressed portion, the **ovary**, and at the apex of a short curved process (the **style**) is a rough yellow surface, the **stigma.** Open one of the carpels carefully, and observe an internal cavity, containing a single round body, the **ovule.**

II. Compare the flower of *Caltha palustris*, the Marsh Marigold. Here the general arrangement of parts will be found to be the same: but note the following points of difference—that the calyx is here petaloid, consisting of five or more sepals, the corolla is absent, and the carpels are fewer (five to ten), but of larger size. Slit open one of the carpels along the dorsal side, turn back the flaps, and observe the **numerous ovules**, attached to the ventral side of the carpel, and arranged in two irregular longitudinal rows.

III. For comparison of the above, examine the greater Stitchwort (*Stellaria holostea*), and note the

regular cymose inflorescence (dichasium). The flower is composed of—

1. Calyx, sepals five, polysepalous, inferior.

2. Corolla, petals five, deeply notched, polypetalous, hypogynous.

3. Andrœcium, stamens ten, polyandrous, or very slightly united at the base, hypogynous.

4. Gynœcium, carpels three, united or syncarpous, superior: styles and stigmas three. Ovary unilocular, ovules numerous, inserted on the prolongation of the axis (central placentation).

This affords an example of **cohesion** of the carpels. Compare the Rose Campion (*Lychnis dioica*), which belongs to the same natural order, and shows the same general characters; but the plants are more or less distinctly **unisexual,** flowers with perfect stamens being borne on some plants, while on others flowers will be found with only the female organs matured: the species is thus **dicœcious.**

Note the calyx with united sepals, gamosepalous: in the male flowers there are ten stamens slightly united at the base, and centrally a **rudimentary gynœcium;** in the female flowers, the gynœcium consists of five carpels, syncarpous and superior, while around its base may be seen ten **rudimentary stamens:** thus this plant illustrates **cohesion** of the sepals and of the carpels, and a partial **suppression** of the stamens, or of the carpels.

IV. Examine flowers of the Bird-Cherry (*Prunus Padus*): they are arranged in racemose manner. Note that each flower consists of—

1. A calyx of five sepals, inserted upon the so-

called calyx-tube, which may be regarded as an enlargement of the floral receptacle.

2. A corolla of five polypetalous petals also inserted on the margin of the calyx-tube, the petals alternating in position with the sepals.

3. Androecium, composed of indefinite stamens, polyandrous, and perigynous, *i.e.* inserted on the margin of the calyx-tube.

4. Gynœcium, consisting of one carpel, superior: ovules two.

This is a typical perigynous flower, in which the sepals, petals, and stamens are inserted on the margin of the calyx-tube.

Compare flowers of Hawthorn (*Cratægus oxyacantha*), which belongs to the same natural order: the number of parts is the same as in the above, excepting that the carpels may be one, two, or three. It differs however in the fact that the calyx-tube is adherent to the ovary, and the ovary accordingly is **inferior.** Compare the Apple.

V. Examine flowers of the Primrose (*Primula vulgaris*), or of the hothouse Primula (*P. sinensis*), and note that it is composed of—

1. Calyx of five sepals, gamosepalous, inferior.

2. Corolla of five petals, gamopetalous, hypogynous, and alternating with the sepals.

3. Androecium of five stamens, which are inserted on the inner surface of the tube of the corolla (**epipetalous**): they are opposite the petals.

4. Gynœcium of five carpels, syncarpous, superior: placentation, free-central, ovules numerous.

This is an example of **cohesion** of sepals, petals, and

carpels, and of **adhesion** of the stamens to the corolla. Note also that the flowers are of two types, short-styled and long-styled (**dimorphic**).

VI. Examine a head or capitulum of the Sunflower: this is an inflorescence, and it is composed of a large number of florets, or small flowers, inserted on a wide, disk-like development of the main axis or peduncle. Note the dark green, closely imbricated **bracts,** which show a gradual transition from the foliage-leaves to a simpler form: these together constitute the **involucre,** which surrounds the margin of the capitulum. On the upper surface of the flattened receptacle are the numerous, and closely packed **florets,** of which two types are to be distinguished—

a. **Ligulate** or **ray-florets,** with broadly strap-shaped yellow corolla, which are disposed at the periphery.

b. **Tubular** or **disk-florets,** which constitute the central part of the head.

In the young inflorescence before flowering, and also later in the fruiting inflorescence, there may be seen opposite, and external to each floret a small leaf (the **bracteole**), lanceolate above, but broadly sheathing below, in the axil of which the floret is produced (compare development, p. 194).

Remove the bracts from the periphery of the capitulum, and separate a single **ligulate floret:** examine it in detail, and observe at the base the more or less compressed **ovary,** which is inferior: at its upper limit is an irregular rim, which may be regarded as representing the **calyx:** above it is the yellow **corolla,** tubular in its lower part, broadly ligulate above: on

slitting the tubular portion there may be seen a more or less reduced style inserted on the apex of the ovary: the stamens are abortive. The florets are thus neuter.

Examine one of the **florets of the disk** in detail, noting first its position in the axil of a bracteole: observe—

1. At the base the laterally compressed **ovary,** which is **inferior.**

2. Seated in an antero-posterior position above it are two chaffy scales, which represent the **calyx,** and it is accordingly **superior.**

3. Above this is the tubular yellow **corolla,** with a throat narrowed below, and terminated by five teeth: it thus consists of five petals, gamopetalous and superior.

4. Projecting from the tube of the corolla may be seen a dark-coloured, cylindrical body, composed of the coherent **anthers,** and projecting through the tube is—

5. The bifid and recurved **stigma.**

Slit the tube of the corolla longitudinally, and note the five separate filaments of the **stamens,** which insert themselves on the inner surface of the narrow throat of the corolla (**epipetalous**), while the anthers are united above so as to form a tube (**syngenesious**).

Remove the corolla and stamens: the long cylindrical **style** will remain rising from the apex of the ovary, and terminating in a **bifid stigma:** thus it is indicated that there are two carpels, syncarpous and inferior.

Open the ovary longitudinally, and note the single

cavity (unilocular), and within it a single anatropous ovule attached to the base of the cavity.

As a substitute, or for comparison, the head or capitulum of the Dandelion (*Taraxacum officinale*) should also be dissected: the general features will be similar to the above, the chief points of difference being that—

1. All the florets are ligulate, and bisexual.
2. The bracteoles are abortive.
3. The calyx is developed as a silky pappus.

For comparison with the above types of flower, which are all Dicotyledonous, examine, as typical of the Monocotyledons, the flowers of the Blue Bell (*Scilla nutans*), which are borne in simple racemes. Each is composed of—

1. A **perianth,** consisting of six petaloid segments which are free, or polypetalous, and hypogynous: three composing an outer whorl, overlap the other three which compose an inner whorl.

2. The **androecium,** consisting of six stamens, each being opposite one of the segments of the perianth to which it adheres.

3. The **gynœcium,** consisting of three carpels, syncarpous and superior: the ovary has three loculi, ovules numerous, placentation axile.

Compare flowers of *Iris*, in which the stamens are only three in number, the ovary inferior, and the stigmas petaloid.

For further details as to the various structure and form of flowers, reference must be made to books on Descriptive Botany.

Development of the Flower.

In order to trace the development of the flower it is found convenient to use plants with **aggregated inflorescences,** *i.e.* those in which the flowers are closely associated together in large numbers. By cutting sections through such an inflorescence many individual flowers, illustrating different degrees of progress, will be traversed, and by comparison of these an idea of the course of development may be gained even from a single section.

I. Examine young **capitula** of the Sunflower with the naked eye: they occur in the same positions as the vegetative apical buds, but differ externally from these—

1. In their greater bulk, and more especially in their diameter being larger.

2. In their colour, which is usually darker.

3. In being covered externally by a large number of imbricated **bracts** (or hypsophyllary leaves), which together form the **general involucre.**

Select a very young capitulum—that is, one in which these characters can be recognized, but are not as yet very pronounced—and, having removed the largest external bracts, cut from it median longitudinal sections: treat with potash for about ten minutes, and mount in glycerine: observe with a low power—

1. That in outline and general arrangement of parts the sections resemble those of the vegetative bud, but that the apical cone is broader, and more flat.

2. That the surface of the cone has an irregular outline, owing to the formation of a series of appen-

dicular organs, which are developed in **acropetal order,** *i.e.* the smallest or youngest are nearest the centre or apex, while on passing towards the periphery the size regularly increases.

Put on a higher power, and study these organs in detail, beginning at the centre.

If the capitulum be young enough, there will be found, as in the vegetative bud, a naked **apical cone,** with a rather flatter form, but a similar arrangement of tissues to that there observed. Passing from the centre, the external surface assumes an undulating appearance owing to the formation of—

1. **Bracteoles,** or small leaves, which arise similarly to the leaves as above observed (p. 82), by outgrowth of the epidermis and subjacent tissue: as they grow older they curve towards the centre. Note the formation of **hairs** of various types from single cells of the epidermis, this being a good opportunity for tracing their origin.

2. The rudiments of **flowers,** which appear in the axils of the bracteoles, *i.e.* on the side nearer the apex. These are likewise produced from the epidermis and subjacent tissue; they are, morphologically speaking, **axillary branches.**

The development of the latter into the complete flower must be carefully studied, by comparison of those nearer the centre with older flowers nearer the periphery of the capitulum, or on capitula of various ages. It is obvious that flowers which have been cut in median section will be best fitted for this study. Note the following successive stages of development—

a. Form of papilla, conical.

b. Apex becomes flattened.

c. Periphery of the flattened apex rises into a whorl of five small lobes; these are the **petals,** which are in the mature flower united as a gamopetalous **corolla.**

d. Between the corolla and the now depressed apex rises a fresh series or whorl of five lobes; these are the young **stamens.**

About this stage may be seen externally, below the corolla, a slight protuberance on each side of the flower (as seen in section): this is the first appearance of the **calyx,** which consists in the mature flower of two scaly sepals.

N.B.—This order of appearance of the floral whorls is not normal, but is the rule in the order *Compositæ.* In the large majority of plants the calyx is developed first, then the corolla, and then the stamens.

e. Within the whorl of stamens there arises, at the margin of the now much depressed apex, the last series of floral organs, viz. two **carpels,** which arch over the apical depression, and thus close in the cavity of the **inferior ovary.**

f. All the organs increase in size, while from the base of the cavity of the ovary a papilla arises, which develops into a single **anatropous ovule,** with one **integument,** and small **nucellus.** (For the details of development of the ovule, see *Caltha,* p. 206.)

II. Cut horizontal (*i.e.* transverse) sections of a capitulum: treat as before: examine with a low power.

Note the arrangement of bracteoles, with young flowers in their axils, round the central naked apex.

The youngest flowers will appear simply circular in outline (simple papillæ of stages *a* and *b*) : older flowers will show successively—

i. The five papillæ of the **corolla** (petals) uniting at an early stage into a gamopetalous corolla-tube. (Stage *c*.)

ii. Five **stamens,** alternating with the petals. (Stage *d*.)

iii. Centrally, **two carpels.** (Stage *e*.)

Other *Compositæ* may be taken as substitutes for *Helianthus*, but some variety will be found in different genera in the character of the bracteoles, and in the calyx: thus in *Chrysanthemum Leucanthemum*, which is a useful type, the bracteoles are absent, and there is no representative of the calyx.

Calyx and Corolla.

Mount in glycerine two small pieces of the corolla of a ligulate floret of the Sunflower which has been kept in spirit, the one with the lower, the other with the upper surface uppermost. Examine under a low power and note, the delicate texture and transparency of the whole: the rarity or even entire absence of stomata: the numerous hairs on the lower, and the small projecting papillæ on the upper surface: and the vascular bundles which do not form a dense network as in the foliage leaf.

Cut transverse sections of the same, mount in glycerine, and observe that the structure is altogether simpler than that of the foliage leaf. Note the smooth lower epidermis: the upper epidermis, of which each

cell has grown out as a **conical papilla**: it is these which give the velvet-like appearance to the corolla: the mesophyll which is very lax, and is not differentiated into palisade and spongy parenchyma.

Sections should also be cut of the sepals and petals of other flowers, such as the Pansy, Pelargonium, Scilla, &c., in order to compare their structure with that of foliage leaves.

The bright colours of flowers and fruits may be due either to colouring matter dissolved in the cell-sap, or to small coloured bodies in the cells, or to both combined. They must be studied in fresh material, as the colourings are altered or destroyed by alcohol. As a first example the common scarlet Geranium (*Pelargonium*) may be taken, in which the petals owe their colour to dissolved matter.

Strip off the superficial tissue from a petal of this plant, and mount in water with the outer surface uppermost: note under a low power the conical form of the superficial cells, and the **bright red colour of the cell-sap**: chromoplasts or formed granules appear to be entirely absent.

The case of the common red and yellow Tulip is a good example of mixed colouring: strip off the superficial tissue from the yellow base of one of the segments of the perianth: mount in water, and examine under a high power: observe the very numerous **yellow chromoplasts** of more or less distinctly crystalline form.

Strip off now a similar patch of tissue from the upper, red portion, mount, and examine as before: here the chromoplasts will be seen as before, but masked by the more prominently coloured **red cell-sap.**

The colouring of fruits is due to a similar cause. From a ripe Tomato remove a small quantity of the inner pulp, and mount it directly on a slide under a cover-slip, or if the pulp be not very deliquescent add a little water.

Note under a low power the pulp, composed of numerous spherical or oval cells, which are more or less completely separate.

Examine a single cell under a high power, and observe the numerous granules (the **chromoplasts**) of an orange or red colour, and irregular form : they are often, like the leukoplasts, grouped closely round the nucleus.

Observations of similar bodies should be made in other brightly coloured fruits, the Rose Hip will be found to be a very good example, and in it the relation of the chromoplasts to the chlorophyll-granules may be observed, by comparing hips in which the colour is turning. They should also be examined in petals of various plants, *e.g.* of *Ranunculus*, *Tropæolum*, and coloured hairs, *e.g.* those on the stamens of *Cucurbita*, &c., &c.

Sections should also be made through the **nectaries,** which occur in many flowers, *e.g.* in *Fritillaria*, *Ranunculus*, &c., in connection with the petals or on the receptacle : these will be found to be essentially similar in structure to, and may be treated in the same way as, the glands described above on p. 142.

The Stamen.

III. All the following preparations should be made from materials hardened in alcohol, or fixed with saturated solution of picric acid, and then washed, and hardened in alcohol.

A. Cut transverse sections of a flower bud of *Caltha palustris*, which was just ready to open, taking care that the anthers shall be cut through transversely. Neglecting the other parts, mount the sections of the **anthers** in glycerine, and examine with a low power. Note:—

1. The general outline of the section, and compare it with the form of the bilobed anther as above observed.

2. The two large cavities or **pollen-sacs** in each lobe.

3. These are surrounded externally by the **wall** of the anther, which consists of at least three layers of cells.

4. The **septa,** which divide the two **pollen-sacs** or **microsporangia** of each lobe from one another; the anther has thus originally four pollen-sacs: these may be found still distinct in almost mature anthers, though as they approach maturity the septa are partially broken down, and the cavities of the pollen-sacs are thus thrown together: this usually happens before the dehiscence of the anther.

5. A single small **vascular bundle** lying symmetrically between the cavities in the central part or **connective** of the anther.

6. **Pollen-grains** or **microspores,** mostly to be found lying free in the glycerine.

Put on a high power and make the following observations:—

1. The wall of the anther is composed of—

(*a.*) A layer of **epidermis,** with an external **cuticle:** within this is—

(*b.*) A layer of cells with a **fibrous thickening** of the cell walls.

(*c.*) Immediately within (*b*) will be seen a narrow highly refractive band, consisting of the remnants of two transitory layers of cells, the inner of which was the **tapetum:** this is almost completely disorganized; the outer of the two layers, which abuts on the fibrous layer, is less completely disorganized, and may be seen as an almost continuous layer of thin-walled cells, even in almost mature anthers. Note also that the wall of the anther is thinnest, and its construction most simple at the part most remote from the connective, *i.e.* where the septum of each lobe meets the wall of the anther, while nearer to the connective it becomes thicker.

2. At the point where the septum meets the wall of the anther the cells are smaller, and of rounded form, owing to the presence of intercellular spaces between them, and the inner layer is not spirally thickened: this is the **line of dehiscence** of the anther, and the lax character of the tissue at this point helps to bring about the rupture.

3. The **pollen-grains** or **microspores,** which are almost spherical, with smooth walls and granular protoplasmic contents, in which may be made out, with difficulty, **two nuclei.**

Treat fresh pollen from a full-blown flower with a solution of methyl-green in 1 per cent. acetic acid, and mount in dilute

glycerine: in pollen-grains so prepared the two nuclei may be found to be stained: better results may be obtained by careful staining with hæmatoxylin.

B. Mount in half glycerine, half alcohol, some almost mature pollen of *Fritillaria imperialis*, which has been previously preserved in alcohol, and examine with a high power. The grains have a smooth wall, and in the granular protoplasm may usually be seen **two nuclei.** If the grains be stained with hæmatoxylin before mounting in glycerine and alcohol, the nuclei will be more easily made out.

Mount and examine, as types of the various forms of the grains, the pollen of *Helianthus*, *Althæa*, *Cucurbita*, *Œnothera*, *Orchis* (pollen-masses or pollinia), *Mimosa*, *Cichorium*, *Rhododendron*, or *Azalea*, &c.

C. In order to observe the germination of the pollen-grains, and formation of the pollen-tubes, cultures may be made in a watch glass, or, if it is desired to follow the process in single individual grains, use may be made of the moist chamber described in Appendix A.

Mount some pollen-grains of *Helianthus* or of *Lilium* in one hanging drop of a weak solution of cane-sugar in water (about 5 per cent.). Examine them with a high power, and note their form and the external configuration of their walls.

Keep them at an ordinary temperature in the dark for 12 to 18 hours: on again examining them, many will be found to have put out **pollen-tubes,** filled with granular protoplasm, in which, after suitable staining, two or more **nuclei** may be detected.

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The same method may be used for the pollen of other plants, *e.g.* Orchids, species of *Tulipa*, *Fritillaria*, *Nymphæa*, &c. It will be found that the time of appearance of the pollen-tube will vary in different cases; also that to obtain good results solutions of sugar of different strengths will have to be used. In most cases a solution of 10 per cent. or less will be found suitable.

Development of Anther and Pollen.

If transverse sections be made from very young buds of *Caltha*, and successively from older ones up to the mature flower, the development of the anther and of the pollen may be traced.

The material should be preserved in absolute alcohol (or strong methylated spirit), and the sections should be treated with half glycerine, half alcohol: this should be left exposed to the air in a watch-glass, so that the alcohol may evaporate: mount in pure glycerine. Anhydrous staining reagents may be employed, but are not actually necessary.

By following this method, sections may be prepared illustrating the division of hypodermal cells by periclinal walls of four points in the young anther, corresponding in position to the four pollen-sacs of the mature one: the outer layer thus produced is the **primary tapetal layer**: the inner is the **archesporium**: tracing the development further, as it may be seen in older anthers, verify the following points:—

i. The cells of the outer, or primary tapetal layer, undergo division by successive periclinal walls into three, and the layers thus produced develop respectively into—

a. The **fibrous layer.**

b. The transitory layer directly within the fibrous cells.

c. The **tapetum.**

ii. The cells of the **inner layer** go to form the **pollen-mother-cells.**

The above points may be ascertained from a comparison of sections of the very youngest anthers with those cut from older ones.

In sections of anthers of medium age and successively of later development the following points are to be observed—

1. The division of each of the pollen-mother-cells into four **special-mother-cells,** by the gradual ingrowth of the wall of the mother-cell.

2. The separation of the members of the tetrads thus formed, and their subsequent development as **pollen-grains.**

3. The gradual disorganization of the **tapetum.**

4. The development of the wall of the anther, as above described, the most marked constituent of it being the fibrous layer.

Compare similar preparations of the young anthers of *Tradescantia,* and note the division of the pollen-mother-cells, without any gradual ingrowth of the wall.

Observe, as far as possible, the divisions of the nuclei of the pollen-mother-cells first into two, then into four ; also the two nuclei in the mature pollen-grain : in order to obtain good results, it will be well to stain with hæmatoxylin, methyl-green, or some other reagent which colours the nuclei.

Carpel and Ovules.

IV. The following preparations must be made from materials hardened in absolute alcohol, or methylated spirit. From an open bud of *Caltha palustris* which has been thus treated, strip off the outer organs, and cut a large number of transverse sections of the **carpels.** Treat the sections with one-half pure glycerine, one-half alcohol, in a watch-glass, and let the alcohol evaporate gradually: pick out those sections which appear to have fairly traversed one or more of the ovules, and mount them in pure glycerine.

Strasburger recommends that in the preparation of such objects the transfer to pure glycerine should be made before the sections are cut.

The sections may be stained with a solution of methyl-green in 1 per cent. acetic acid: this will give good results, but all the contents of the embryo-sac can be readily seen in good sections without any staining at all.

Examine first with a low power, and observe—

1. The **carpel,** having a structure not unlike that of an ordinary leaf, and consisting of an upper and lower epidermis with some four layers of mesophyll between them. Note the **suture** or junction of the two margins of the carpel, which thus incloses a central cavity.

2. The **ovules** (**macrosporangia**) seated in this cavity, and attached near the margins of the carpel: it has already been noted that there are two rather irregular rows of ovules in each carpel, therefore at most only **two** ovules appear in each section.

The form of the ovule is **anatropous**: it consists of the following parts:—

a. The **funiculus,** or stalk, which adheres through the greater part of its course (as the **raphe**) to the body of the inverted ovule. A procambium bundle, connected with a bundle at the margin of the carpel, traverses it longitudinally.

The body of the inverted ovule consists of—

b. Two **integuments,** each several layers of cells in thickness, the outer being united with the funiculus: the integuments cover the body of the ovule completely, excepting a narrow channel (**micropyle**) near its apex. Within the integuments lies—

c. The **nucellus,** an oval mass of cellular tissue in which is embedded—

d. The **embryo-sac** (**macrospore**), a large oval cell,

situated centrally a short distance below the apex of the nucellus.

Examine the embryo-sac with a high power, and observe—

1. The granular, vacuolated protoplasm which fills it: embedded in this are to be found—

2. A large central **nucleus,** with highly refractive **nucleolus.**

3. At the micropylar end of the embryo-sac, **three cells,** with clearly defined nuclei. Two of these (the **synergidæ**) fill the apex of the sac, the third (the **ovum**) being placed laterally, a little below the apex.

4. At the posterior end of the sac are three cells (the **antipodal cells**), also with clearly defined nuclei. Divisions of these cells occasionally occur, so that their number may be found to be greater than three.

Note the **tapetum,** consisting of cells more or less disorganized, which partially or completely surround the embryo-sac.

If similar sections be cut from buds of *Caltha palustris* of various ages, and be treated in the same way, the development of the ovule, and more especially of the embryo-sac, may be followed, and the various stages of it may be observed.

Make similar sections of the ovary of species of *Lilium*, or *Yucca*, and compare them with the above. With the exception of certain minor points, the structure of the ovule will appear to correspond to that of *Caltha*.

FERTILIZATION.

I. Remove from flowers of *Stellaria media,* which have just faded, the three styles: moisten them with alcohol, and mount quickly in water: note the cylindrical

colourless **styles,** curved at their upper ends: the **stigmatic surface** with its numerous papillose hairs is found on the **convex** side of the curved part of the style. Note especially the numerous yellow **pollen-grains** adherent to the stigmatic surface, while it may often be seen that a **pollen-tube** will proceed from the pollen-grain, and enter the tissue of the style.

II. Cut median vertical sections through the stigma and upper part of the style of a flower of *Datura Stramonium* which has just faded. Mount in dilute glycerine, and examine first with a low power. Note—

1. The closely packed tissue covering the **stigmatic surface,** the superficial cells of which are slightly elongated perpendicularly to the surface as hairs.

2. The more lax **cortical tissue** of the style, with numerous intercellular spaces, which appear dark under the microscope.

3. A central band of more transparent tissue without intercellular spaces (**conducting tissue**).

4. Small vascular bundles, two in number, running up the style: these may or may not be present in the section, according to the direction in which it has been cut.

5. **Pollen-grains** adhering to the surface of the stigma: from them **pollen-tubes,** similar to those grown in sugar solutions (cf. p. 202), may often be traced penetrating the tissue of the stigma.

Now gently boil the sections in the dilute glycerine over a spirit-lamp, and examine again. Observe—

1. The **pollen-grains** as before.

2. **Pollen-tubes,** which may be traced from them through the now more apparent tissues of the style: they may be recognized by their densely granular contents.

Other flowers besides the above may be used, *e.g.* species of *Œnothera, Digitalis*, &c., or any flower in which the style and stigma are of considerable size.

III. The style and stigma of flowers of *Rhododendron ponticum* from which the corolla has already fallen off will also be found to be good material for showing pollen-tubes. Cut transverse sections of the style in the fresh state, mount in dilute glycerine and observe—

1. The tissue of the style with small vascular bundles dotted in it.

2. The **star-shaped central cavity,** filled with mucilage, embedded in which may be seen—

3. The small **pollen-tubes** cut transversely, and embedded in a mass of transparent mucilage.

Cut longitudinal sections of the same, including the stigma, and mount as before : observe—

1. The irregular **stigmatic surface.**

2. The numerous **pollen-grains** (associated in groups of four) attached to the stigma, and often putting out pollen-tubes which penetrate the tissue of the style.

3. The **pollen-tubes,** often to be seen as a dense sheaf, pursuing their course down the cavity of the style : note their thin walls, and the presence of highly refractive **plugs,** which stop their cavities : look for endings of the tubes, in which the protoplasm will be denser, and one or two nuclei may be observed there.

IV. Pick out gently a number of ovules from an ovary of a flower of *Datura Stramonium*, or of *Digitalis purpurea*, which has just faded, and mount in dilute glycerine. Observe—

1. The **campylotropous ovules,** with curved body.

2. **Pollen-tubes,** which are often to be found with the end applied closely to the micropyle.

Similar observations may also be made on *Stellaria media,* and many other plants.

Strasburger observed the process of fertilization itself directly in *Torenia asiatica, Gloxinia,* and also in Orchids, *Monotropa,* and *Pyrola.* His method was to open the ovary of a flower a short time after pollination, and detach and mount the ovules in a 3 per cent. solution of sugar.

Results of Fertilization.

A. Development of the Embryo.

i. *Dicotyledon.*

Pick out the ovules from a fresh ovary of *Capsella Bursa-pastoris,* which has attained about half the ultimate size of the mature fruit: material kept in spirit will not do well for this work. Treat with dilute potash, and examine with a low power. Observe—

1. The form of the ovule (**campylotropous,** *i.e.* with a curvature of the body of the ovule).

2. The **funiculus,** or stalk.

3. The **integuments.**

4. The **micropyle,** not very easily seen: a **pollen-tube** may often be observed entering the micropyle.

5. A large central cavity (the **embryo-sac**), which is curved like the whole ovule. In this may be seen, more or less distinctly—

6. The **embryo.**

To study the structure of the embryo, either longitudinal sections of the ovule must be cut, and the embryo

be thus laid bare, or the embryo must be removed from the ovule. The former is the more accurate method, though the latter is much the easier: the latter will therefore be adopted.

Press gently with a needle upon the cover-slip of the above preparation, so as to burst the ovules: the embryo will escape in some cases without injury; but this will only be the case when fresh material has been used; after hardening in alcohol the embryos will not readily leave the ovule. Neutralize the potash with dilute acetic acid. The structure of the embryos, which now lie freely suspended in the fluid, may be easily studied.

Apply the same method for the preparation of embryos, from ovaries of various ages, both younger and older than that first taken. A series of preparations may thus be obtained illustrating various stages of development of the embryo, such as are figured in ordinary text-books.

Note more especially the following successive stages of development:—

1. The **suspensor,** consisting of one or more cells and terminated by a single **embryonic cell.**

2. The embryonic cell divided into **octants** arranged in two tiers: the suspensor is elongated and the cells divided so as to form a series, of which the basal cell (that nearest the micropyle) is usually enlarged greatly, so as to exceed the embryo in size, and beginners are apt to mistake it for the embryo: the terminal cell next the embryo (the **hypophysis**) encroaches between the four lower octants of the embryo.

3. The octants so divided as to form three layers

of cells, which have been distinguished as (*a*) the external **dermatogen,** (*b*) the **periblem,** (*c*) the central **plerome.**

4. The two **cotyledons** formed by lateral outgrowth from the upper tier of octants, the apex of the **radicle** derived from the hypophysis, the hypocotyledonary stem from the lower tier of octants.

5. Other parts as before. The **apical bud** or **plumule** formed between the cotyledons.

ii. *Monocotyledon.*

Treat ovules of *Alisma Plantago* in the same way, and observe the following stages of development:—

1. **Suspensor** and **embryo** consist of a single short series of cells, produced by transverse divisions.

2. The terminal cell divides longitudinally into four (first tier).

3. The second, third, and fourth cells from the end also divide successively (second, third, and fourth tiers).

4. The cells of the body of the embryo divided (as in *Capsella*) so as to form three layers: (*a*) external **dermatogen,** (*b*) **periblem,** (*c*) central **plerome.**

5. A lateral depression of the surface, at the level of the second tier. At the basal lip of this the **apical cone** of the plumule is formed.

The single **cotyledon** is formed from the first tier.

The **radicle** from the third tier.

The **apex** of the root from the fourth tier.

Compare these results with those obtained in *Capsella.*

B. DEVELOPMENT OF THE ENDOSPERM.

I. This may be traced in the embryo-sac of *Caltha palustris* in material which has been fixed and preserved in absolute alcohol or strong methylated spirit: it is an advantage to collect the material on a hot day, and place it in alcohol without delay; by this means many nuclei may be fixed in various stages of division.

Cut transverse sections of the carpels of a flower of *Caltha palustris* which is full blown, or even beginning to fade, and also sections of successively older specimens up to the almost mature fruit: treat them as before described (p. 204), and compare them: they may illustrate the changes which appear in the embryo-sac subsequently to fertilization, viz. :—

1. The penetration of the micropyle and apex of the nucellus by the pollen-tube.

2. The first stages of development of the embryo, which in this case remains relatively small, the seed being an albuminous one; the embryo will thus be seen *in situ*.

3. The division of the central nucleus of the embryo-sac into two, subsequently into four, eight, &c.

4. The disposition of the nuclei, as they increase in number, as a dense series embedded in the protoplasmic film at the periphery of the embryo-sac.

5. The formation of cell-walls between these nuclei, so that the embryo-sac is lined internally by a single layer of cells of the endosperm.

6. The ingrowth of these cells, and their subsequent

division so as to fill the cavity of the embryo-sac with endosperm which embeds the embryo.

7. The great increase in size of the embryo-sac, and of the whole ovule.

8. Note also the changes in the integuments, and the disappearance of the nucellus as the ovules become mature.

On looking over a number of such sections, numerous cases of **division of nuclei,** illustrating various stages of the process, may be found. These points may be very well studied in the embryo-sacs of *Fritillaria imperialis*, &c.

II. For obtaining preparations of the embryo *in situ*, and of the **endosperm** surrounding it, the ovary of species of *Potamogeton* will be found to be good material: it should be previously hardened in spirit.

Cut longitudinal sections of a single carpel, parallel to the flattened sides. Mount in glycerine, and examine with a low power. One of the sections will probably be found to include—

1. The **embryo-sac,** in which are contained—

2. The **embryo,** with a very short suspensor, the basal cell of which is greatly enlarged.

3. The **endosperm,** a tissue which lines the embryo-sac, and varies in appearance according to the stage of development of the ovary.

III. The continuity of protoplasm through cell-walls has been shown in the sieve-tubes (p. 115). Similar observations of fine threads of protoplasm traversing the cell-walls may be made in the endosperm of various seeds, and these are merely prominent examples of a wide-spread phenomenon.

Cut thin sections with a dry razor from the endosperm of the dry seed of *Strychnos Nux-vomica:* first mount a section in glycerine, and observe, under a high power, the dense protoplasmic body of each cell surrounded by a thick cellulose wall.

Mount other sections in *tincture of iodine not diluted with water:* then place a small drop of water at the edge of the cover-slip, drawing it under by means of blotting-paper: observe the edges of the section where the effect of the dilution will first appear, and as the cell-walls swell, it will be seen that they are traversed by fine threads of protoplasm, which are stained by the reagent.

Another method has been found to succeed well in demonstrating continuity through the cell-walls of the endosperm of various Palms, in which the endosperm has thick pitted walls consisting of reserve cellulose: it is, to immerse the fresh sections in sulphuric acid in which a small quantity of powdered Hoffmann's blue has been dissolved: when the sections are sufficiently acted upon, wash them with water and mount in glycerine. The protoplasm will be stained a deep blue, and the swollen cell-wall is not stained: examine the swollen pit-membranes, and if the treatment has been successful they may be seen to be traversed by fine curved strands of stained protoplasm.

The tissues of the cortex of *Rhamnus* have been found to be good material for the demonstration of continuity of protoplasm.

MATURE SEED AND EMBRYO.

(A) *Dicotyledons.*

I. (*a*) SOAK some Broad Beans for 24 hours in water: selecting one which is fully swollen, note its flattened form, and the dark blotch (the **hilum**) at one edge of it: this is the base of the seed, by which it was attached to the parent plant. Dry the surface of the seed and squeeze it gently, water will be seen to exude from a small hole close to the hilum: this hole is the **micropyle,** and is a guide to the position of the technical apex of the seed, the whole being of the curved or campylotropous type.

Remove the tough outer **seed-coat,** derived from the integuments, and the bulky, yellowish **embryo,** which occupies the whole space within the seed-coat, will then be disclosed: it is to be observed that there is here no tissue derived from nucellus, and no endosperm, and the seed is therefore described as **exalbuminous**: note the following parts of it:—

1. The two fleshy **cotyledons,** which are attached to one another at one point, which is their base.

2. The conical **radicle,** which lies externally, and in the seed has its pointed apex directed towards the micropyle.

3. Separate the two cotyledons, and between them observe a bud, the **plumule,** composed of numerous small plumular leaves.

(*b*) Compare with the Bean the flattened seed of the Cucumber or Gourd: the micropyle may be found by the same means as before, at the pointed end of the

seed, and close to it is the small scar of the hilum : this seed is of the anatropous type and is **exalbuminous.** Peel off the leathery seed-coat, and note the parts of the straight embryo, viz.—

1. The radicle directed towards the micropyle.
2. The two cotyledons, fleshy as before.
3. Between them the very small plumule.

Very similar results will be obtained from the Almond, in which, however, the testa is the relatively thin brown covering. Compare also the Walnut, in which the cotyledons are corrugated, and the seed of the Sycamore, in which they are spirally rolled together, &c.

(*c*) Examine the ripe fruit of the Sunflower (*Helianthus annuus*). The "seeds" sold for sowing are really fruits (achænia), including the products of development of both ovary and ovule. It is a dry inferior achænium, with narrower basal, and broader apical end : at the latter is a scar, where were inserted the style and other floral organs.

Compare fruits *in situ* on the floral receptacle.

Dissect off the brittle **pericarp,** from the anatropous and exalbuminous seed, which it incloses.

Note the delicate **seed-coat,** and, within this, the straight **embryo,** of which the **radicle** is directed towards the micropyle (*i.e.* towards the base of the fruit), and the two **cotyledons** towards the apex of the fruit.

Separate the two cotyledons, and note between them, at their point of attachment together, the minute **plumule.**

(*d*) Compare the structure of the **albuminous** seed of the Castor oil (*Ricinus communis*), observing externally

the hard, bright, variously marked **seed-coat,** which has attached to it at the basal end a wart-like swelling —the **arillus.**

Remove the testa, which is brittle and easily cracked; note beneath this a thin papery white layer,—the *tegmen* of old writers: this closely invests the white oily mass of the **endosperm,** a tissue which is not present at the period of maturity in any of the seeds above described. Cut this through transversely: and a flattened central cavity will be found, lined on either side by one of the thin flattened **cotyledons** of the embryo. Lay open the endosperm of another seed longitudinally, by a cut following the plane of the flattened cavity: it will then be clearly seen that the straight embryo is embedded in a mass of endosperm, and that it consists of two cotyledons, radicle, and plumule.

(*e*) Examine also the soaked fruits of *Mirabilis:* these are invested by a thick rugged brown coat, which is the indurated and persistent base of the **perianth:** the pointed end is the apex, the flattened end the base. Remove this outer coat, and the light-brown **achene** will be found covered by a thin double investment which represents the **pericarp** and **seed-coat:** through this the form of the curved embryo can be distinguished, especially the **radicle,** at the tip of which (*i.e.* at the base of the achene) will be seen the scar or **hilum,** while at the opposite or upper end a little brown wart represents the insertion of the **style.**

Remove the pericarp and seed-coat, and observe the **embryo** curved round, and inclosing a central mass of **mealy endosperm.** Recognize the parts of the

embryo as before, viz. two flattened cotyledons, the cylindrical radicle, and very small plumule.

Longitudinal sections should be cut of the mature seed of *Caltha* for microscopic observation under a low power, and the relative positions of the firm testa, the endosperm, and embryo, are to be observed, together with details of their structure.

FRUIT, SEED, AND EMBRYO.

(B) *Monocotyledons.*

II. Soak fruits of the Maize (*Zea Mais*) in water for several hours. The fruit is a caryopsis, and results from the development of both ovule and ovary; its form is compressed conical, the apex of the cone being the basal point of attachment of the fruit.

Strip off the external coat of the fruit: this represents both the **wall of the ovary** and the **integument** of the ovule.

Distinguish in the body of the fruit which remains—

1. A lateral, smaller, white portion: this is the **embryo.**

2. A larger yellow part, which forms the greater mass of the fruit: this is the **endosperm.**

Separate the embryo from the rest, and note its shape.

III. Cut longitudinal sections of the fruit so as to traverse the embryo in a median plane: mount in glycerine, and examine with a low power: observe—

i. The coat of the fruit, consisting of two layers, the

pericarp, and **seed-coat.** Note at the apex of the fruit the remnant of the **style,** and the scar of attachment at the base.

ii. The **endosperm,** consisting of thin-walled parenchyma; the cells contain polygonal starch-grains, embedded in a matrix of protoplasm: in the peripheral yellower portion of the endosperm the starch-grains are more closely packed than in the central whiter portion.

iii. The **embryo,** which is in close apposition to the endosperm: the part which is in contact with it is the **scutellum;** it extends over the whole surface of contact, and almost completely surrounds the body of the embryo itself. Note the central attachment of the scutellum to the body of the embryo; the vascular bundles, which form a connection through it; the **epithelium** of peculiar structure, which faces the endosperm.

The body of the embryo consists of—

a. An **apical bud,** with several sheathing leaves, which surround the apical cone.

b. A **radicle,** having similar arrangement of the meristem to that of the older root (compare p. 183). Outside the radicle, and continuous with the root-cap, is a root-sheath, or **coleorhiza:** note that the body of the radicle is covered externally by a clearly marked series of cells which gives rise to the piliferous layer, and being enclosed by the coleorhiza, it is not continuous with the superficial epidermis of the shoot.

IV. For comparison, examine the "stone," or seed, of the Date. Distinguish the basal end, at which the remains of the funicle may still be seen, from the upper more or less pointed end of the anatropous ovule: note an irregular longitudinal fissure running up one side

while the other side is almost smooth: about two-thirds down the smooth side a small circular depression will be seen: this indicates the position of the **embryo.**

Cut the seed transversely so as to traverse the embryo, and observe the external brown **seed-coat,** which closely invests the hard pearly mass of **endosperm,** in which is embedded the relatively very small **embryo.**

RESERVE AND TRANSITORY MATERIALS IN SEEDS, TUBERS, &c.

I. **Starch** from the potato tuber has already been described with its reactions on p. 44—47: as further examples of its occurrence in seeds, cut sections of the cotyledon of the Pea or Bean: stain with iodine solution, and observe the large, oval starch-grains distributed through the tissue: note at the same time the protoplasmic matrix, and numerous small aleurone grains, which are stained yellow or brown.

Cut sections also of the endosperm of the Maize, and treat with solution of iodine. Note the polygonal starch-grains (blue), and the protoplasmic matrix (brown).

Leukoplasts, or **Starch-forming Corpuscles.**—These are to be found in colourless tissues, in which a storage of starch is taking place; *e.g.* a young Potato, the rhizome of *Iris*, or *Canna*, &c.

Cut up very young Potatoes into small pieces, and treat with picric acid: wash out carefully with dilute alcohol, and harden.

Cut sections parallel to the surface, and not far below it (since the leukoplasts are best seen in the most superficial cells immediately within the corky rind): treat the sections for a few minutes in alcohol with a few drops of iodine solution added; or

in very slightly diluted tincture of iodine, and mount in pure glycerine: examine under a high power, and observe—

1. The cells of the usual parenchymatous type, with protoplasm and nucleus.

2. Numerous spherical bodies, usually aggregated round the nucleus: in some cells these will stain blue (starch), with a small yellowish body attached which is the leukoplast; in other cells, which were nearer the outer surface of the tuber, the bodies will stain uniformly yellowish brown: these are the young **leukoplasts** which have not yet formed starch.

The material which has been found best suited for the observation of leukoplasts is the young tuber of the Orchid *Phajus grandifolius* (*Bletia Tankervilliæ*), but it is not to be expected that this will be within the reach of all. Here the leukoplast is of large size, and rod-like form: this material may be treated similarly to the Potato, or a method recommended by Strasburger may be adopted, viz., to cut the sections and fix and stain them with picro-nigrosin, which colours the leukoplasts a steel-blue tint.

II. For **inulin** and its reactions see p. 119.

III. **Cellulose** occurs as a reserve in the endosperm of the Date, and other Palms: the appearance of these has been described on p. 38, and the sections should be cut and the reactions noted. Thickened cell-walls will also be found in sections of the cotyledons of *Lupinus*.

IV. **Sugars.**

(*a*) Grape-sugar. Cut a transverse section of a ripe grape, of such thickness that some cells at least shall be uninjured: mount in water, and observe under a low power the transparent parenchymatous pulp, consisting of cells with thin walls, very sparing contents, and large central vacuole.

Treat for a few minutes with a relatively large bulk of alcohol in a watch-glass: on re-examining, numerous crystals will now be seen in the cells.

Irrigate thoroughly with water: the crystals may be seen to be re-dissolved: they consist of **grape-sugar,** which is in solution in the cell-sap of the living cell.

2. Squeeze out the juice of some Grapes into a test-tube: add a little of Fehling's solution (see Appendix A), and boil: a bulky yellow precipitate is formed owing to reduction of the copper.

3. Soak a fairly thin section of a Grape in Fehling's solution: wash quickly with water, mount in water, and boil gently over a spirit-lamp: a precipitate like the above (2) is formed: note under the microscope that the dark-looking granules of the precipitate (cuprous oxide) are to be found actually within the cells of the tissue, thus indicating that the sugar was there.

(*b*) **Cane-sugar.**

1. Cut transverse sections of the Beet-root: mount them in water, and note under a low power the transparent tissue, and coloured cell-sap. Treat such a section for a few minutes with alcohol in a watch-glass: on re-examination under the microscope, crystals will be seen in the cells, but of smaller size than in the Grape. Re-dissolve by irrigation with water.

2. Boil some small pieces of Beet-root in a small bulk of water: pour off the coloured extract, add to it a little of Fehling's solution, and boil: no precipitate will be formed.

3. This point may be further verified by testing sections as directed above, under head (3): no precipitate will be formed either in the cells or in the surrounding fluid.

V. **Oil-drops.**

Cut thin sections of the cotyledons of the Almond:

mount in water, and note the bright-looking oil-drops, both in and about the section, and dispersed also in the water.

1. Irrigate with alcohol: the drops are not dissolved.

If sections of the oily endosperm of *Ricinus* be treated thus, the oil will be dissolved: this is characteristic of ethereal oils, but is as a rule not the case with the fixed oils: to this the oil of *Ricinus* is an exception.

2. Treat a section with a considerable bulk of ether in a watch-glass: wash with alcohol, and mount in alcohol, or in glycerine: on examination the oil will be found to have been dissolved by the ether.

3. Stain a thin section with tincture of alkanet (see Appendix A), the oil-globules stain pink.

4. Treat a section with 1 per cent. solution of osmic acid: the oil-drops will stain slowly, taking a dusky or black hue.

5. Treat a section with potash solution, and warm: the oil will be partially and slowly saponified and dissolved. This effect of potash is best seen in specimens where the oil is present only in small quantities as isolated globules.

VI. **Aleurone-grains.**

These are found of the largest size in oily seeds: they vary somewhat in their characters in different plants: those of *Ricinus* may be taken as a good type.

Having noted the hard, variously marked **testa,** with the wart-like swelling (aril) at the basal end, crack and remove it: beneath it will be found the white oily mass of the **endosperm.** Cut this through transversely, and with a razor wetted with olive oil, or castor oil,

cut thin sections from it, and mount in the oil. Examine under a high power, and observe—

1. The thin cell-walls of the oval cells.

2. The numerous highly refractive **aleurone-grains** in each cell; each is of oval form, and a less highly refractive area is seen at one end: this is the **globoid.**

3. The oily matrix in which the grains are embedded, this being so transparent as to be hardly visible.

Cut other sections with the razor wetted with alcohol, soak them well in alcohol in a watch-glass to dissolve the oil out of them (ether is a more ready solvent of the oil, and may be used instead of alcohol: wash off the ether with alcohol), and mount in pure glycerine: examine them under a high power, and observe the appearance of the aleurone-grains as before when seen in oil.

i. Add water gradually, and watch its effect on the grains.

1. The outer **amorphous coat** of the grain will swell, and become less highly refractive: thus there will be disclosed—
2. The **crystalloids,** one (or rarely more) being included in each grain: these do not swell greatly with water, and accordingly they retain their refractive power.
3. The **globoid** will also be visible as before.

ii. Add dilute potash solution: the amorphous coats and the crystalloids will swell and dissolve, leaving the globoids.

iii. Add strong acetic acid: the globoids will dissolve

slowly. As regards further reactions of aleurone-grains see Appendix B.

VII. **Crystalloids.**—Crystalloids of cubical form are to be found in the superficial tissues of the Potato. Cut tangential sections from material hardened in alcohol, or in picric acid and alcohol, and mount in pure glycerine and iodine: the cubical crystalloids will be distinguished by their yellowish brown staining.

Treat a section, in which one or more crystalloids are under observation, with potash: the crystalloids will be seen to swell and dissolve.

Mount another section in alcohol or in glycerine, and, having observed the crystalloids, irrigate with a saturated solution of common salt: this also will dissolve the crystalloids.

GERMINATION.

(A) *Dicotyledons.*

(*a*) Examine seedlings of *Helianthus* which have been germinating for different periods from one day to one week, and observe the following points in the process of germination :—

1. The internal parts of the fruit swell, and cause the brittle pericarp to split longitudinally.

2. The radicle protrudes, and curves downwards.

3. The hypocotyledonary stem elongates, so that the pericarp and seed-coat are carried upwards by the cotyledons, which remain inclosed by them for a considerable time.

4. The coats of the fruit fall from the cotyledons,

which soon turn green, and expand as assimilating leaves, with the plumule seated between them.

5. The plumule develops leaves, which expand in succession.

6. The radicle has meanwhile elongated, and produced lateral roots.

Notice that when the young root is removed from the soil many particles adhere to it, especially at some distance from the apex: these are held by the **root-hairs** which attach themselves closely to the particles of soil.

(*b*) With the above compare seedlings of *Ricinus* in various stages of germination: in the main features the results are the same, but note especially that the endosperm remains for a long period in close connection with the cotyledons, and that as the seedling grows that tissue loses its firmness and density, owing to the abstraction of the nutritive substances stored in it, and their transfer through the cotyledons to the seedling.

(*c*) It will be found useful to compare the germination of other seeds also, *e.g.* the Broad Bean, Kidney Bean, Cucumber or Gourd, &c.

The internal changes accompanying the process of germination, and more especially the redistribution of the reserve materials stored in the embryo, may be studied by cutting sections of the seedling at different stages of the process, and comparing the cell-contents in the corresponding tissues. Note especially the corrosion of the starch-grains, in those cases where starch is stored in the seed.

(B) *Monocotyledons.*

I. Comparing plants of Maize which have been germinating for different periods, the following facts in the history of germination may be observed:—

1. The fruit swells.

2. The outer coat ruptures opposite the apex of the radicle, which soon protrudes, bursting through the coleorhiza also, which appears as an irregular ring round the base of the young root. Since the coleorhiza is thus burst through by the young root, it is clear that the epidermis of the shoot is not continuous with the piliferous layer of the root.

3. The rupture of the coat extends upwards to the point opposite the apical bud, which also emerges.

4. The root elongates, and forms lateral roots: other lateral roots (usually two) burst out above the insertion of the scutellum: these soon equal the primary root in length, hence there is no well marked tap-root.

5. Leaves of the plumule unfold, and gradually turn green: the leaf inserted lowest, which was the outermost of those composing the plumule, remains small and develops no expanded lamina: this is the **cotyledon**, according to Hofmeister and other writers.

II. From a young plant with leaves about three inches long, cut longitudinal sections so as to traverse the whole fruit and the contiguous part of the seedling in a median plane: mount in water, and irrigate with solution of iodine. Observe—

1. That in the neighbourhood of the surface of the

scutellum the starch-grains are in course of demolition, and that the central part of each is first attacked.

2. That no starch-grains are to be seen in the epithelium of the scutellum.

Seeds of the Date should also be sown, and the process of germination followed: it may then be seen that the cotyledon, which remains in contact with the endosperm, exhausts the substance of it, gradually increasing the cavity in which the embryo originally was: meanwhile the body of the embryo, with plumule and radicle, is pushed out from the cavity, owing to the increase in length of the cotyledon.

Asparagin.

This substance is found in most seedlings, but in specially large quantity in those of *Lupinus luteus*. Cut sections from fresh seedlings, and, after a preliminary examination of them, irrigate gradually with alcohol: the substance is precipitated in the form of crystals.

Sections of material which has been kept in alcohol, mounted in glycerine, will show these crystals already formed, and often of large size. These sections should be irrigated with water; the crystals will dissolve.

The most distinctive test is a saturated solution of asparagin: if this be added to sections containing crystals presumably of asparagin, they will not be dissolved, whereas crystals of inulin would be dissolved.

II. GYMNOSPERMS.

VEGETATIVE ORGANS.

EXTERNAL CHARACTERS.

A. Examine a plant of the common Norway Spruce Fir (*Abies excelsa*), and observe that while the **main axis** grows vertically upwards, and bears pseudo-whorls of **lateral branches,** which radiate from it as a centre, the branches themselves are ramified only in a horizontal plane: the branches are thus **dorsiventral,** having distinct upper and lower surfaces.

The branches as well as the main axis bear numerous simple, linear, four-angled **foliage-leaves,** which may however have dropped off from the older parts, leaving clearly-marked scars. Note also at the bases of the lateral branchlets, and at irregular distances along the relative main branch or the main axis itself, that there are zones where dry chaffy **scale-leaves** were inserted: these protected the winter-buds when young, and their bases remain persistent: the portions between successive zones represent the annual increments of growth in length, and, as the lateral branches are for the most part inserted immediately below these zones of scales,

it follows that they are produced at the upper end of each annual increment.

Examine the buds at the apices of the stem and branches: they are covered with brown scale-leaves: thus a distinction is to be drawn between protective scale-leaves, and the foliage-leaves.

There is considerable variety in the character of the non-reproductive leaves of the *Coniferæ*, though the form of the individual leaf is simple throughout the series. In some cases only green foliage-leaves are developed, as in *Araucaria*, *Juniperus*, *Thuja*; in *Phyllocladus* only scale-leaves are produced, while the flattened stems assume the assimilative function; in other cases, as in *Taxus* and *Abies*, there is an alternation of scale-leaves which protect the bud, and foliage-leaves; in *Pinus sylvestris*, the somewhat complicated shoot of which will be described in detail below, there are both scale- and foliage-leaves, the former alone being borne on the stronger axis of unlimited growth, while the latter (together with scale-leaves) occur only on the foliage-shoots of limited growth (bifoliar spurs). Specimens of different genera of the *Coniferæ* should be examined and compared.

B. Take a branch of *Pinus sylvestris*, cut in autumn, including at least four years' growth.

N.B.—The limits of each year's growth may be recognized externally at those points where false whorls of strong lateral axes are developed; and the portion of stem lying between two such whorls may be regarded as roughly representing one year's growth.

I. Consider first the growth of the year in which the branch was cut, *i.e.* the part above the youngest whorl of lateral axes. At its apex is a large **bud**, surrounded by a variable number of smaller **lateral buds.**

From a bud, which has been treated with alcohol to

remove the external secretion of the resin, detach some of the brown **scale-leaves,** which cover it externally. Note—

1. The succulent base of these scales.
2. Buds in their axils.

Compare these winter-buds with some of the same which have been cut in late spring. The brown scale-leaves will be found to have fallen off, leaving their succulent bases still persistent; in the axils of these will be seen the axillary buds above noted. The main axis of the bud has become elongated by extension of the tissues.

In studying the growth of the current year, bear in mind that it has been derived from a bud which had a similar structure to that which is now seated at its apex. Examine the stem of the current year externally, and note—

1. The thick **main axis,** more or less succulent in appearance: its surface is marked by longitudinal grooves.

2. The brown tooth-like bases of the scale-leaves of the bud, best seen at the lower part of the internode.

3. In the axils of these, especially at the upper part of the internodes, are **axillary buds** of two kinds.

a. **Buds with limited growth (bifoliar spurs),** each bearing two acicular **foliage-leaves,** surrounded at the base with numerous scale-leaves. These bifoliar spurs occur in the axils of the scales throughout the greater part of the current year's growth: in older parts they may be found to have fallen off, the bifoliar spurs separating as a whole from the parent branch.

b. **Buds with unlimited growth,** which are seated

close to the apex of the shoot of the current year. They are few in number: their structure has already been observed: each may develop into an unlimited axis.

It may here be observed that both (*a*) and (*b*) have a similar origin, both being axillary buds in the axils of the leaves of the main axis of the current year. The apparent difference depends upon the fact that the buds (*b*) are more strongly developed than (*a*).

II. Passing to the increments of growth of former years, *i.e.* to the lower and older parts of the branch, in the external appearance and arrangement of parts they resemble that of the current year. The main axis increases in thickness, and is more obviously ligneous, while the bifoliar spurs drop off, leaving scars which mark their former position.

THE STEM.

It is best to work with material which has been treated for some time with spirit; by this means the resin, which would otherwise clog the razor, is removed.

I. Cut transverse sections of the **axis of a bud,** and treat with dilute potash for a few minutes: mount in glycerine.

Meanwhile other sections may be mounted in chlor-zinc-iodine: examine with a medium or low power, and observe at the centre of the section—

1. The **pith,** composed of cells with intercellular spaces, and thick cellulose walls (blue with chlor-zinc-iodine): surrounding this a series of groups of smaller constituents: these are—

2. The primary **vascular bundles.** Note that they

are separated from one another laterally by bands of parenchyma; that their form is approximately wedge-shaped; and that the tissues of which they are composed may be distinguished as—

i. **Xylem,** nearer the centre of the stem, the components of which have thick, dark-looking, lignified walls (yellow with chlor-zinc-iodine). These first-formed xylem-elements, since they differ from those formed later, are distinguished as **protoxylem.**

ii. **Phloem,** nearer the periphery, with bright-looking cellulose walls (blue with chlor-zinc-iodine).

The more minute study of these tissues must be deferred for the present.

Outside the ring of vascular bundles is—

3. The **cortical tissue,** a mass of cells similar in structure to the pith. In this occur large intercellular spaces, which are **resin-passages.** Since the periphery of the section of the axis of the bud is complicated by great irregularity of outline, the study of the outer tissues will be better carried out in the older stem.

II. Cut transverse sections of the stem of the current year: mount some in glycerine, others in chlor-zinc-iodine: the sections have a wavy outline, the indentations corresponding to the grooves above observed externally. Starting from the periphery of the section, note the following tissues:—

1. **Epidermis,** a single layer of cells, following the wavy outline of the section: the walls, especially the outer, are much thickened: externally there is a **cuticle.**

2. **Cortical tissue,** consisting of cells with rather thick cellulose walls (blue with chlor-zinc-iodine), and

protoplasmic contents with chlorophyll. Many cells have recently divided: this is necessary to keep pace with the growth in thickness of the vascular cylinder. Large intercellular spaces (**resin-passages**) occur here and there, and are lined with small-celled epithelium. (Compare p. 65).

It must be remembered that in the present case the resin itself has been dissolved out by alcohol: sections should, therefore, be made from fresh material in order to see the secretion *in situ*. It appears amorphous and transparent: it is soluble in alcohol, leaving a slight residue.

The secretion stains deeply with tincture of alkanet. (See p. 65.)

Near the periphery of the cortex will be found a layer of **cork** and a **cork-cambium** (compare stem of Elm, p. 91), derived from cells of the cortex by their division by tangential walls. The cells of the cork have no cell-contents: their walls are coloured yellowish brown with chlor-zinc-iodine.

Treat a section with strong sulphuric acid. The walls of the cork retain their sharp contour.

At the bases of the indentations at the margin of the section, and immediately below the epidermis, note groups of sclerenchyma, having thick lignified walls (yellow with chlor-zinc-iodine).

3. The **vascular system** is here a complete ring, though it is composed of separate bundles in the bud (see above, p. 232): distinguish as before, the external **phloem,** the internal **xylem,** and the misty layer of **cambium.**

The vascular bundles were seen to be separated in

the bud by intervening parenchyma: here the ring has been completed by the formation of an **interfascicular cambium** in the parenchyma between the original bundles. (Compare p. 99.)

Observe that the internal limit of the vascular ring is sinuous: the convexities mark the position of the primary bundles: at the inner limit of these will be found the **protoxylem.**

4. The **pith** consists of parenchyma, having the same characters as in the bud: there are no resin-passages.

Put on a high power, and examine the **cambium.** Note—

i. That the cells are arranged with great regularity in **radial rows.**

ii. That their walls are thinner than those of the surrounding tissues, and are composed of cellulose (blue with chlor-zinc-iodine).

iii. That the tangential walls are thinner than the radial.

iv. That the cells have copious protoplasm, in which a nucleus may often be recognized.

These facts point to a repeated division of cells by tangential walls. (Compare Fig. 9, A, p. 99.)

Draw carefully, and compare several of the radial series of cells of the cambium. They will be found to coincide with Sanio's law of cambial division, which was first concluded from observations on *Pinus sylvestris.*

Observe, here and there, radial rows of which the cells are more elongated in a radial direction than the rest: these may be traced outwards towards the cortex

and inwards towards the pith: they are the **medullary rays.** (Compare Fig. 9, A, row 2.) Some of them may be traced the whole way to the cortex and to the pith (primary medullary rays), others only part of that distance (secondary medullary rays).

Note that the cells of the medullary rays at the cambium-zone are less elongated radially than in the xylem or phloem, the cambium being the formative region of the rays as well as of the other tissues.

The mature cells of the ray usually have cellulose walls (blue with chlor-zinc-iodine), and granular protoplasmic contents with nucleus. In fact the cells of the medullary rays usually retain their cell-nature.

Follow the radial rows of cambium-cells outwards, and note the gradual transition to the permanent tissues of the **secondary phloem,** the constituents of which are also arranged in radial rows, and have cellulose walls (blue with chlor-zinc-iodine). The ring of secondary phloem is cut up into rectangular areas by the **medullary rays,** which are easily recognized as above directed. Observe that the tissues filling these areas are of three sorts—

i. Elements with cellulose walls, and no very distinct contents: they are radially compressed: these are the **sieve-tubes,** which compose the greater part of the phloem. The walls are differentiated into layers, and have bright globules attached to them.

ii. Here and there the radial rows of sieve-tubes are broken by single large cells of the **bast-parenchyma,** which resemble in their characters those of the medullary rays.

iii. Towards the periphery of the phloem are elements

similar in form to the sieve-tubes, whose cell-contents are brown, and contain crystals.

Note on passing to the periphery of the phloem an increasing irregularity of form of the tissues, due to distortion, caused by pressure from without by the cortical tissue upon the vascular system, as it increases in bulk by secondary thickening.

Sclerenchymatous elements are absent from the phloem of the stem of *P. sylvestris*. They are, however, found in the phloem of many of the *Coniferæ*, *e.g. Juniperus*, in which the different tissues are arranged with great regularity.

Follow the radial rows of cambium-cells inwards, *i.e.* towards the centre of the stem. Note the transition from thin-walled cambium to the thick-walled tissue of the **xylem.** If the stem was cut in winter the transition will appear sudden, if cut in summer it will appear gradual. The tissue-elements retain the same arrangements in radial rows, as the cells of the cambium.

Observe that the xylem-ring is cut by the medullary rays into wedge-shaped areas. The chief tissue-elements filling these areas are the **tracheides,** which present the following characters :—

i. They have approximately the same shape as the cells of the cambium from which they are derived.

ii. Their walls are thick and lignified (yellow with chlor-zinc-iodine), and are differentiated into layers, distinguished optically, and by staining.

iii. They have no cell-contents.

iv. On their **radial walls** (and rarely on the tangential walls) are found the **bordered pits,** which are

best seen in the xylem formed at the early part of the year.

These appear, when seen in transverse section under a low power, as bi-convex enlargements of the wall, which look darker than the rest of the wall : under a high power it is seen that there is a biconvex-lens-shaped **cavity of the pit,** over-arched on either side by a meniscus-like outgrowth of the wall, which is seen in good sections to be perforated at its centre : the cavity of the pit is traversed by the thin **pit-membrane,** enlarged at its central point, and occupying either a median position, or more commonly arched to one side : the wall is thus not perforated at the pits, but they are due to irregularity of thickening of the wall, the thinnest part remaining persistent as the pit-membrane.

Observe near the centre, and bordering on the pith, the **protoxylem** arranged as above observed in the younger stem. No bordered pits occur in the walls of the protoxylem.

Note the occurrence of **resin-passages** in the secondary xylem, lined as before by thin-walled epithelium, which may be regarded as a form of **xylem-parenchyma.**

III. Cut transverse sections of a **three-years-old stem** so as to include the whole width of the vascular ring ; it is not necessary, however, to have a complete transverse section of the whole stem. Mount in glycerine. Comparing this with what has already been observed in the stem of the current year, note the following differences :—

1. The cortical tissue bears evident traces of tangen-

tial extension. This is necessary to keep pace with the increase in bulk of the vascular system.

2. The phloem is thicker, and the constituents of the outer part of it are much distorted and displaced.

3. The xylem has increased in thickness more than any other tissue, so that it is now the chief constituent of the stem. It may be distinguished as being composed of three bands (**annual rings**), in each of which the more central tracheides have large cavity and thinner walls (wood developed in spring); passing outwards through the annual ring there may be seen a gradual reduction of the cavity, and increase in thickness of the walls till a certain limit is reached (autumn wood). Outside the latter is a sudden transition to the spring wood: at this point is the limit of each year's growth.

IV. Cut radial longitudinal sections of a three-years-old stem: mount some in glycerine, others in chlor-zinc-iodine.

The sections should be accurately radial and longitudinal, otherwise the difficulty of study of the tissues is greatly increased.

Beginning at the centre of the stem and passing outwards, observe successively:—

1. The **pith,** consisting of two sorts of elements, both of which are of parenchymatous form.

a. Cells with pitted cellulose walls, and having protoplasm and nucleus.

b. Elements of similar form with pitted lignified walls, and no cell-contents.

From the arrangement of these it may be concluded that they had a common origin.

2. The **xylem,** consisting of—

a. **Tracheides** with lignified walls, and no cell-contents. Starting from those nearest the pith, and passing outwards, the following forms may be observed, and distinguished mainly by the markings due to unequal thickening of the walls.

i. Tracheides with narrow cavity, and more or less regular annular or spiral marking—the **protoxylem.**

ii. Elements wider than these, and with bordered pits scattered between the spirals.

iii. Normal **tracheides,** with bordered pits only: these form by far the greater bulk of the secondary xylem, and must be carefully studied. Their form is **prosenchymatous.** The greater part of the cell-walls is of uniform thickness. On these portions of the wall observe with the high power two intersecting systems of **lines of striation.** In single longitudinal rows are found the **bordered pits:** each of these has the appearance of two concentric rings, of which the smaller is more strongly marked, and corresponds to the opening of the cavity of the pit into the cell-cavity, the larger represents the limit of area of the pit. It must be remembered that we are now observing the radial walls in surface view. A careful comparison should be made of the bordered pit as seen here in surface view with its appearance when seen cut through, as in the transverse sections above described.

Note the **annual rings** recognized here as in the transverse sections; the autumnal wood being distinguished by the smaller size of the cavity, and greater thickness of the walls of the tracheides.

b. Here and there the continuity of the mass of tracheides is broken by a longitudinal **resin-passage,** surrounded by parenchymatous cells, which have cellulose walls and retain their cell-contents.

c. The whole mass of xylem is traversed radially by plates of parenchyma (**medullary rays**). Note that they extend only a short way longitudinally, but a long way radially; also that they are composed of cells arranged like bricks in a wall, among which may be distinguished—

i. Cells with cellulose walls, and protoplasmic contents: the pits in the walls of the tracheides which abut on these are unusually wide.

ii. Elements with no protoplasm, and with lignified walls marked with bordered pits.

Both tissue-forms may often be found in the same ray, though rays will often be seen consisting of (ii) alone. Note that between the cells (i.) there may commonly be seen clearly defined lines running radially: these are **intercellular spaces.** (See below p. 243).

3. The **cambium-layer,** consisting of elongated thin-walled cells, the ends of which are difficult to observe. They have copious protoplasm, and an elongated nucleus. (Compare Fig. 9, B, p. 107).

Note that the medullary rays are continuous through the cambium, and observe the differentiation from

the uniform cambium of the ray to the forms (*a*) and (*b*).

In the sections through the cambium of a stem cut in summer, the development of the bordered pits on the walls of the tracheides may be studied.

4. The **phloem** tissues, which are best studied in sections which have been treated for some hours with chlor-zinc-iodine, consist of—

a. **Sieve-tubes,** elongated structures with cellulose walls, those which are radial being marked by numerous circular **sieve-plates,** here seen in surface view: these sometimes stain a sherry brown with chlor-zinc-iodine. The ends of the tubes are difficult to observe: their protoplasmic contents are transparent and sparing.

b. **Phloem-parenchyma,** cells arranged in longitudinal rows, with cellulose walls, and copious protoplasm.

c. Occasional elements (prosenchymatous or parenchymatous) with brown cell-contents, in which **crystals** are embedded: these are found towards the periphery of the phloem.

Medullary rays will be seen with a similar arrangement to that seen in the xylem. Their cells, which resemble those of the phloem-parenchyma in character, are all alike.

5. Externally to the phloem is the **cortical parenchyma,** which requires no further notice here. Outside this is cork, and at certain points a little sclerenchyma. At the periphery of the section is the **epidermis.**

V. Cut tangential sections of a three-or four-years-old branch, and bear in mind that as a rule the central part

of the sections is the most accurately tangential, *i.e.* that the plane of section is there most accurately perpendicular to the radius of the stem. (See p. 8). Sections should be cut at different depths in the tissues, so that the middle of the plane of section shall traverse (A) the peripheral part of the xylem, (B) the cambium, and (C) the inner part of the secondary phloem. Mount as before.

A. In sections which pass through the peripheral part of the xylem observe—

i. The **tracheides,** of prosenchymatous form. No bordered pits (or very few) are seen in surface view, but they may be seen in large numbers in the radial walls (here cut longitudinally), presenting a similar appearance to that seen in transverse sections. (See p. 238).

ii. **Medullary rays,** which resemble a section of a biconvex lens. Note that each ray extends only a short distance in a longitudinal direction: in some cases rays consist of only a single radial series of cells, of which only one lenticular cell appears in this section. In those rays which consist of several rows of cells, note the small triangular **intercellular spaces** which intervene between the cells: these are only seen in thin sections. Occasionally a **resin-passage** is included in a ray.

iii. Longitudinal **resin-passages.**

B. In sections passing through the cambium will be seen—

i. The **cambium-cells,** resembling the tracheides in form (prosenchymatous): the cell-walls are thin, and the protoplasm granular, with elongated nucleus.

ii. The **cambium of medullary rays** is similar in

shape to the cells of the rays: it is thin-walled, with granular protoplasm and nucleus. (Compare Fig. 9, C, p. 107.)

If these sections be treated with dilute potash, and mounted in glycerine, their structure may be more easily made out.

C. In sections passing through the phloem will be seen—

i. The **medullary rays** as before, but their form is more convex: all the tissues between the medullary rays are derived from cambium-cells of the form above observed. These are—

ii. **Sieve-tubes,** which retain the form of the cambium-cells: the cellulose walls seen in surface view are smooth: those cut longitudinally appear of wavy outline (sieves): the structure of the latter is well seen after treatment with chlor-zinc-iodine for twenty-four hours. The contents consist chiefly of transparen protoplasm.

iii. **Bast-parenchyma,** derived from cambium-cells by their division by transverse walls.

iv. Some few cells, especially towards the periphery containing **crystals** which give the reactions of calcium oxalate.

THE LEAF.

Examine the bifoliar spur of *Pinus sylvestris* as a whole: it consists of a very short axis, at the base of which are borne membranous sheathing scales, and at the apex two long needle-shaped foliage-leaves: in other species of *Pinus* the number may be larger. Note that

the inner, or morphologically upper surface of the leaf is flat, while the outer or lower surface is rounded, and the whole leaf is traversed from end to end by two sharp ridges which are slightly rough to the touch.

Cut transverse sections of a foliage-leaf taken from a stem of the current year. It may be found convenient to embed in paraffin, or to hold the leaf between pieces of pith, or carrot. Mount some in glycerine, others in chlor-zinc-iodine, and examine with a low power. Note the semilunar form of the section: the flat side is the upper, the convex side the lower. Starting from the periphery observe successively the following tissues:—

1. A single layer of **epidermal cells** with very thick walls: enlarged cells are to be found at the two corners, and since these cells project slightly they cause the roughness above noted.

2. A narrow band of thick-walled **hypoderma.**

3. A broad band of chlorophyll-containing **mesophyll,** with resin-passages.

4. A **bundle-sheath** or **endodermis,** consisting of oval cells.

5. A broad band of tissue without chlorophyll (the **pericycle**), which surrounds—

6. Two central **vascular bundles.**

Study these several tissues under a high power.

1. The **epidermal cells** have their thick walls differentiated into three layers. These may be recognized without staining, or better after treatment with chlor-zinc-iodine, as—

i. A thin external **cuticle,** not very deeply stained: it extends as wedge-like processes between the cells.

ii. The **cuticularized layers,** forming a thick band,

which stains a deep brown. Immediately surrounding the cell-cavity is—

iii. A broad pitted band, not deeply stained. Compare the epidermis of the Holly, p. 132.

This differentiation may be brought into greater prominence by treating with strong sulphuric acid, or by staining slightly with fuchsin. If sections are boiled for ten minutes or more in strong solution of potash, i. will be dissolved, while ii. and iii. remain.

Note the larger cells at the angles of the section, with thicker walls.

Here and there depressions of the external surface may be observed. These indicate the position of the **stomata.** Observe the two **guard-cells,** which are seated some distance below the surface of the leaf: an intercellular space (respiratory cavity) is to be seen immediately below each stoma.

2. The **hypoderma** (sclerenchymatous) varies in thickness from a single layer of cells to several layers. It is thickest at the corners of the section: the cells are thick-walled, and lignified. Note that it is absent below the stomata.

3. The **mesophyll** consists of thin-walled, chlorophyll-containing parenchyma: the cellulose walls (blue with chlor-zinc-iodine) show a peculiar in-folding. **Resin-passages** occur in it: their cavity is lined with thin-walled epithelium, which is immediately surrounded by a layer of thick-walled sclerenchyma.

4. The **endodermis** has its walls stained brown with chlor-zinc-iodine.

5. The tissue immediately within this, which may be called the **pericycle,** consists of two elements—

i. **Parenchymatous cells,** with thin cellulose walls (blue with chlor-zinc-iodine), and protoplasmic contents.

ii. Elements having lignified walls, with bordered pits, and no cell-contents (tracheides, or transfusion-tissue).

6. The two central **vascular bundles,** the constituents of which resemble those of the stem. Note that the xylem is directed towards the upper surface. Thick-walled sclerenchyma is scattered irregularly round the bundles. It will thus be seen that the leaf of the Pine is of the centric type.

Compare the foliage-leaf of the Yew (*Taxus*), noting its flattened form and short petiole: the upper surface is convex, the lower concave, and there is a clearly marked midrib.

Cut transverse sections, and observe in them the convex upper surface, covered with an epidermal layer without stomata: there is no hypoderma, and resin-passages are absent from the mesophyll: the latter consists of palisade-parenchyma immediately below the upper epidermis, and spongy parenchyma towards the lower.

The epidermis covering the concave lower surface has peculiar excrescences of the outer wall of the epidermal cells, and numerous stomata. Centrally is a single vascular bundle, with the xylem directed upwards. Thus this leaf is distinctly of a bifacial type.

THE ROOT.

I. Cut transverse sections of a young primary root of the seedling of *Pinus* (not necessarily *P. sylvestris*): treat with dilute potash, or "eau de javelle," and mount in glycerine. Observe—

1. A thick band of **cortex,** with a superficial, but ill-

defined piliferous layer (compare longitudinal sections of the root: below, p. 251.)

2. The **endodermis** at the inner limit of the cortex: this is a single layer of cells, having the characteristic marking on the radial walls. Within this lies—

3. The **pericycle**, a band three or four layers of cells in thickness, immediately surrounds—

4. The central **vascular cylinder,** in which may be seen—

a. Y-shaped groups of **xylem,** the fork of the Y directed outwards; their number varies (3–6): between the limbs of the fork of each lies a **resin-passage.**

b. Groups of **phloem,** equal in number to the xylem-groups, and alternating with them: these tissues of the phloem are not very easily recognized.

c. Centrally is a mass of **parenchyma,** which also extends between the xylem and phloem and separates them from one another.

II. Cut other sections from an older part of the root, and treat as before. Observe that—

1. The cortex becomes disorganized and brown.

2. Divisions appear in the outermost cells of the pericycle, forming a layer of cork.

3. Lateral roots may occasionally be found, originating in the pericycle, opposite the xylem.

4. The parenchyma lying centrally to the phloem-groups has begun to divide as a **cambium-layer.**

III. Cut transverse sections of a thin lateral root (about $\frac{1}{32}$ of an inch in diameter) of a full-grown tree of

P. sylvestris: mount some sections in glycerine, others in chlor-zinc-iodine. Observe successively, starting from the periphery of the section—

1. Withered remnants of the **cortex:** this may, however, have been already completely thrown off.

2. The **pericycle,** with its secondary products, consisting of a thin external band of **cork,** the cells of which are arranged in radial rows: within it is the **cork-cambium,** having the same characters as above described (p. 92): internally will be found the remainder of the original pericycle in a quiescent state.

3. The **phloem,** forming, according to the age of the root, a more or less complete ring. The constituents resemble those of the phloem of the stem, and are often distorted by pressure of the external tissues.

4. The **cambium,** as in the stem.

5. The **xylem,** in which may be recognized, near the centre—

a. The groups of **primary xylem,** arranged in the form of a Y, and each having, as before, a resin-passage in the fork: the number of these is usually three (triarch), or four (tetrarch).

b. The masses of **secondary xylem,** more or less fan-shaped, and alternating in position with the groups of primary xylem. The number of the latter, and of the masses of secondary xylem, varies in the lateral root, four being the average number. The constituents of the secondary xylem resemble those of the stem in structure and arrangement.

IV. Cut, and mount as before, transverse sections of a root about one-eighth of an inch in diameter.

The arrangement of tissues will be as before, but the

fan-shaped masses of secondary xylem will have joined laterally, so as to form a complete ring. **Annual rings** may also be seen—in fact, the structure at the periphery of the root now closely resembles that of the stem after it has undergone secondary thickening.

The root of *Taxus* differs in certain details of structure from that of *Pinus*, and as it is typical of the majority of Gymnosperms, and is an excellent type for illustrating the process of secondary growth in thickness, it will be well to make preparations from it also: the specimens are to be dug up carefully, and washed to remove the soil. Selecting a young fibril of succulent appearance, cut transverse sections at a point not far removed from the smooth apex: treat with "eau de javelle" or potash, wash with water, and mount in glycerine. Examine under a lower power, and observe—

1. The **piliferous layer,** not clearly defined, while numerous superficial cells have grown out as **root-hairs.**

2. The broad band of **cortex**: note especially the well-marked layer of cells, next outside the endodermis, with a peculiar thickening of the radial walls; sometimes the thickening extends to cells of the next outer layer, and in some other Conifers (*e.g. Cupressus*) it may be found to extend over several layers.

3. The **endodermis,** which lies immediately within this layer, and consists of cells with thin brown walls, and with the usual dark dot on the radial cell-walls. Within this is the vascular cylinder, which consists of—

4. The **diarch, primary xylem,** of which the two originally distinct groups join at the centre to form a diametral plate.

5. The two masses of **primary phloem,** on either side of this, are not well marked.

6. Between the vascular tissues and the endodermis is a bulky **pericycle,** which usually consists of only a single layer opposite the xylem, but it widens out into several layers opposite the phloem.

Cut transverse sections of an older part of the root: note in these the cortex as before, or it may be turned brown, and be

partially disorganized: the cells of the endodermis will be distorted by pressure from within: a brown corky tissue, varying in thickness according to age, will be seen to have been derived from the outermost layer of the pericycle. Centrally the diametral plate will be seen as before, and the cambium on either side of it will have formed secondary xylem internally, and secondary phloem externally: note the primary phloem squeezed out of shape by the increasing bulk of the internal tissues.

Cut transverse sections of a still older root $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter, and these will show the cortex thrown off entirely, and the phloem, which is not clearly marked nor largely developed, surrounded by the pericycle, with its peripheral layer of cork. Internally the xylem will be seen, marked with annual rings as in the stem, but centrally the diametral plate of primary xylem may still be recognized.

V. Cut median longitudinal sections of the apex of the root of *Pinus*. This may be most easily done by cutting longitudinal sections of the mature embryo in the seed. Treat with potash or "eau de javelle" till they are transparent, and mount in glycerine. Observe—

1. The central **plerome-cylinder,** recognized as in the Sunflower and the Maize. It is rounded off at the apex, and throughout is quite distinct from—

2. The **periblem,** which surrounds it: this is the formative tissue of the cortex: outside this no true epidermis is to be found, but at the apex is—

3. A **root-cap,** which is formed by the active division of the cells of the periblem at the apex of the root.

Compare this arrangement of the apical meristem with those types seen in the roots of Angiosperms (see p. 157, 183).

REPRODUCTIVE ORGANS

It has been above noted (p. 231) that at the apex of the ordinary vegetative branch in spring there is an apical bud surrounded by a number of lateral buds, all of which normally develop into vegetative axes of the type above described. The reproductive organs of *Pinus* are produced on buds corresponding in position to these: they are easily recognized, even at an early stage of development, with the naked eye.

The following observations should be made upon specimens preserved in alcohol, otherwise they could only be made at intervals, according to the period of development of the organs in question.

A. **Male inflorescence.**—Note that the inflorescence while young appears as a bud covered with brown **scale-leaves,** in the axils of which are **lateral axes** easily seen on removing the scales. Of these lateral axes—

a. Those nearest the apex of the bud develop as lateral foliage-shoots, as is the case on the ordinary vegetative axis.

b. Below these, a number bear, in place of the two foliage-leaves, numerous **staminal leaves**: to each one of these axes the term **flower** may be applied.

Comparing the male inflorescence with the ordinary vegetative axis, the main difference lies in the mode of

development of the lateral axes. In autumn the male inflorescences of the preceding summer can only be distinguished from the purely vegetative axis by the absence of the lateral foliage-shoots from the lower parts of them.

I. Separate a single male flower, and cut it longitudinally in a median plane: it will be found to consist of—

1. An **axis,** which bears—
2. A number of **staminal leaves.**

Detach some of these staminal leaves with a needle: each consists of—

a. A short stalk, or **filament,** which bears at its apex—

b. An expanded **anther,** with two swellings on the lower surface (**pollen-sacs,** or **microsporangia**).

II. Cut longitudinal sections of the male flower in which the pollen is not yet ripe, and mount in glycerine: examine with a low power. Note the arrangement of the parts as above described: in the pollen-sacs note the **pollen-grains** *in situ* (**microspores**).

The pollen is ripe about the middle of June, and material should be collected and preserved in alcohol at short intervals during May and June, so as to illustrate various stages of development. By cutting sections from such material, and treating as above directed, the history of development of the pollen may be made out.

III. Mount ripe pollen-grains (*i.e.* such as may be collected by shaking a male branch in June) in dilute glycerine, having previously wetted them with alcohol. Observe—

1. The two large lateral **wings,** usually filled with

air, which facilitate the carriage of the pollen by the wind: these are extensions of the outer coat (extine).

2. The central body of the pollen-grain, consisting of—

a. A large cell, which constitutes the greater part of the grain, and from which the pollen-tube springs.

b. A series of one or more smaller cells affixed laterally to the wall of the pollen-grain at a point between the wings: they are placed on the convex side of the grain, which is not so completely covered by the wings. These take no direct part in the formation of the pollen-tube.

B. **Female branches or cones.**—Observe on a Scotch Fir, towards the end of June, that there are cones to be found in three different stages of development, the position of which is constant.

a. **Small green cones,** one or more of which occur close to the apex of the shoot of the current year. Note that the basal part, or stalk, bears brown membranous scales, while the upper part is globular, and is marked out into numerous square areas, which are the apices of the **ovuliferous scales.**

Comparing a shoot, which bears such young cones, with an ordinary vegetative shoot, it will be seen that the cones correspond in position to the lateral buds, of which they are the morphological equivalent.

b. **Larger green succulent cones,** which occur laterally at the apical part of the shoot of the previous year: the arrangement of parts on these corresponds to that on (*a*).

c. Cones larger than (*b*), brown and with lignified tissues: on these the scales are usually more or less

separated from one another, so as to disclose the **seeds,** two of which are borne at the base of each of the ovuliferous scales. These ripe cones are seated laterally near the apex of the two-years-old shoot.

I. Cut median longitudinal sections of a cone corresponding to stage (*a*). It should previously have been hardened with alcohol for some days: mount in glycerine, and examine with a low power. Observe—

1. The central **axis,** not differing essentially from the young vegetative axis: on this are borne scales of two orders easily distinguished by their size.

2. The **smaller** of these are the leaves borne by the axis of the cone, and the morphological equivalents of the brown scale-leaves which cover the winter buds.

3. In the axil of each of these is borne one of the larger or **ovuliferous scales,** which are longer and more bulky than (*b*): they alone can be seen externally. On the upper surface of each of these, close to the axis, are borne—

4. Two **ovules,** or **macrosporangia,** which are anatropous, so that the micropyle is directed towards the base of the scale: if cut in a median plane, each ovule will be seen to consist of—

i. One **integument,** several layers of cells in thickness, with a widely open **micropyle** facing the axis: this surrounds—

ii. The **nucellus,** a mass of parenchyma, near the centre of which is—

iii. The **embryo-sac** or **macrospore,** a cell much larger than those of the surrounding tissue, and lying some distance below the apex of the nucellus.

Pollen-grains may often be found seated on the apex of the nucellus: one or more of these may throw out **pollen-tubes,** which penetrate into its tissue.

Dissect off one whole ovuliferous scale, and observe on its upper surface, close to the base, **two ovules,** which are anatropous. Note also the relative positions of the two sets of scales.

II. Take cones of the stage above described as (*b*).

The material should be collected about the middle of June, and must be hardened in alcohol.

Strip off the ovuliferous scales of such cones: the ovules will remain adherent to the base of each. Cut longitudinal sections of the scales so as to pass through the median planes of the ovules: mount in pure glycerine, and examine with a low power. Observe—

1. The structure of the **ovuliferous scale,** which is traversed by vascular bundles, and resin-passages.

2. The **ovule,** which is united with the scale, and consists, as in the younger stage, of—

a. An external **integument:** note the wide **micropyle.**

b. The **nucellus** as before, but larger.

c. The **embryo-sac,** filled with the thin-walled tissue of the **endosperm.** All the parts of the ovule are larger than in the younger stage, but retain the same relative positions. Note carefully that **pollen-grains** (one or more) are usually to be found lying on the apex of the nucellus, and that from the larger cell of each of them arises a cylindrical **pollen-tube,** which traverses the tissue of the nucellus as far

as the apex of the endosperm, where it widens out into a large sac.

Observe near the apex of the endosperm, and embedded in it, one or more large vacuolated protoplasmic bodies; these are the **egg-cells,** or **ova:** from the apex of each may be traced a narrow **neck** or channel, inclosed by smaller cells than those of the surrounding endosperm. The neck and central cell together form the **corpusculum,** that is, the **archegonium.** The endosperm corresponds to the prothallus of the Fern, while the corpuscula are equivalent to the archegonia.

III. Remove ovules from cones of the second year, taken and preserved in alcohol about August 1. Dissect off from them the now hardened **integument** or **seed-coat:** note within this the delicate remnant of the **nucellus,** which covers the mass of **endosperm.** Soak the latter in water, and dissect from it with needles the numerous **embryos,** which lie in the central cavity of the endosperm: treat them with potash, and mount in dilute glycerine. Examine with a low power, and observe—

1. The **suspensors,** coiled filaments consisting of numerous transparent thin-walled cells. At the ends of the suspensors are borne—

2. The **embryos:** they are more or less elongated, almost cylindrical bodies: in some cases (only one as a rule in each seed) they may have already formed—

a. An **apical cone,** which terminates the free, anterior end of the embryo; this being surrounded by—

b. A whorl of **cotyledons** of variable number.

c. The apex of the **radicle,** directed towards the suspensor (*i.e.* towards the micropyle of the ovule), and embedded in the tissue at the posterior end of the embryo.

Note that there is no definite boundary between the suspensor and the embryo. Also that though **polyembryony** is the rule—that is, a number of embryos are at first formed simultaneously—one of these supersedes the rest, and that one alone becomes differentiated as above described.

By cutting sections of ovules taken between the dates above named, and treating them in the manner described for the cones taken in June, the history of the early stages of development of the suspensors and embryos from the fertilized ovum may be traced.

Ripe Seed.

Examine the ripe seed of *P. sylvestris,* or other species, *e.g. P. pinea,* and note the external hard and thick **seed-coat:** within this the **endosperm,** which incloses the single **embryo.** It has numerous **cotyledons,** and a **radicle,** the apex of which is directed towards the micropyle.

Germination.

Compare plants in different stages of germination, and observe the following points in the process:—

1. The endosperm swells, and bursts the testa.
2. The radicle protrudes, and curves downwards.
3. The cotyledons elongate, and push out the stem,

and their own basal portion, from the cavity of the endosperm.

4. The rest of the seed is usually carried upwards on the apex of the cotyledons, which, with the hypocotyledonary stem, elongate greatly.

5. The plumule develops, forming numerous acicular leaves.

Note that the cotyledons turn green while still protected from the light, below the soil, and within the testa.

Compare the reproductive organs of the Yew (*Taxus baccata*): in this plant pollination takes place late in March, and the seed attains maturity in the course of the same year.

Examine male flowers in early spring, and observe—

1. Their axillary position.

2. The **scale-leaves,** which are inserted at the base of the short **axis.**

3. The **staminal leaves,** which occupy the upper part of the axis, each bearing a peltate head with 4–5 **pollen-sacs.**

Moisten a little of the pollen with alcohol, and mount in water: observe the simple form of the grains, and the absence of the wings.

Examine also the female flowers, which are produced on different plants from the male organs, the species being dicœcious: they appear as solitary axillary buds, consisting of a single central, and apparently terminal **ovule,** surrounded by a number of small imbricated scales: the micropyle may be easily recognized at the apex of the ovule, and at the receptive period a sticky drop may be seen to have exuded from it.

Cut median longitudinal sections through one of these ovules, taken late in March, and observe the axis (a secondary lateral axis) terminated by the **nucellus**: this is surrounded by a single **integument** with an obvious micropyle: a single larger cell,

deeply seated in the tissue of the nucellus may be recognized as the **embryo-sac**, or macrospore.

Cut similar sections from specimens taken later in the year: in these the growth of the embryo-sac, and development of its contents, as well as the growth of the pollen tubes, the process of fertilization, and development of the embryo may be traced.

Note the growth of the **arillus**, as an extra integument, pink and fleshy when mature, around the base of the ovule.

The observations of structure and development of the ovule may be best conducted in material hardened in alcohol, and then soaked, before cutting, in half glycerine, half alcohol.

PTERIDOPHYTA

A.—LYCOPODINÆ

I.—Heterosporous Type

SELAGINELLA MARTENSII

SPOROPHYTE

I. In a well-grown plant note with the naked eye the following external characters :—

1. The **stem** ascending, frequently branched, apparently in a dichotomous, but really in a monopodial manner (see p. 270): the branching occurs only in a single plane.

2. The **leaves,** of small size, and simple in form, with a ciliate margin, and arranged in alternating pairs : each pair consists of a dorsal and a ventral leaf, the whole series thus forming **four orthostichies :** note the two different sizes of leaves—

a. The **larger ventral** leaves, arranged in two orthostichies, and without terminal awns.

b. The **smaller dorsal** leaves, also arranged in two orthostichies, each leaf being terminated by a fine **awn.**

Each leaf has a single central nerve or **midrib.** Turn back one of the leaves, and observe with a lens the small scale-like **ligule:** note that the insertion of each leaf is oblique.

3. The **rhizophores,** long cylindrical branched organs, which arise at the points of branching of the obliquely ascending stem, and grow vertically downwards: note their frequent bifurcations. Two rhizophores are formed at each branching of the axis, one on the dorsal, and the other on the ventral side; of these only the latter is as a rule developed beyond the first rudimentary stage.

Remove a rhizophore, which has grown down so as to reach the soil, and wash it: observe—

4. The delicate **roots,** which rise at the point where the rhizophore touched the soil, and branch in a monopodial manner: and though they often seem to bifurcate it appears not to be a case of true dichotomy.

Observe further that many of the branches of the stem may have a symmetrical arrangement of the leaves close to the apex: these are the branches or **cones,** which bear the **sporangia:** note that on these cones—

i. The leaves are all similar to one another and of small size.

ii. That they are arranged in four symmetrical orthostichies.

iii. That, on turning the leaves back, one **sporangium** will be disclosed in each case. On comparing a number of sporangia which have been exposed in this way, it may be seen that there are two sorts of them—

a. **Macrosporangia,** which are of a green or light-brown colour, and appear to be of rounded tetrahedral form.

b. **Microsporangia,** which are more nearly spherical, and of a reddish-brown colour.

Note in older cones that the sporangia are already open, **dehiscence** having taken place in a plane parallel to that of the leaf.

II. Cut out as thick a piece of the stem as can be found, and about one inch in length: note a central white dot on the transversely cut surface: this is the single central **vascular bundle.** Slice off the upper surface of the stem with a razor till the whole course of the vascular bundle is laid bare, and observe with a lens—

1. The course of the central vascular bundle, which is directly longitudinal and median.

2. The smaller lateral bundles, which pass from the central bundle without branching, into the leaves, and traverse the midribs of the leaves.

III. Cut transverse sections of a well-developed stem: mount some in glycerine, others in chlor-zinc-iodine: others again may be mounted in acid solution of aniline sulphate. Examine first under a low power, using a high power when necessary, and observe the following tissues in succession, starting from the periphery of the section:—

1. At the periphery a layer of small, thick-walled cells, forming an ill-defined **epidermis,** with no stomata: it is covered externally by a continuous **cuticle.** Beneath the epidermis, and not clearly marked off from it, is—

2. The **cortical tissue :** the cells of the peripheral part of it have thick stratified and lignified walls, with no intercellular spaces. Passing inwards there is seen a gradual decrease in thickness of the walls, and increase in size of the cells, till an abrupt limit is reached at—

3. The **lacunar tissue,** consisting of thin-walled cells, which form irregular **trabeculæ** traversing the intercellular cavity in a radial direction: the inmost cells of these trabeculæ have a peculiar equatorial constriction.

This lacunar tissue is more typically represented in some of the larger species, *e.g. S. inæqualifolia, S. Willdonovii,* &c. It may be regarded as the equivalent of the **endodermis** of most other vascular Cryptogams.

4. By means of these trabeculæ the single central **vascular bundle** is suspended in the middle of the large air-cavity: the bundle is of elliptical outline as seen in the transverse section, and is built upon the **concentric** type: it is composed of the following tissues:—

a. The **pericycle,** an irregular band of comparatively large, thin-walled cells, which completely surround the central tissues, and abut externally on the intercellular cavity, and the trabeculæ. The cells of this layer, in common with all the outer tissues, including the epidermis, may contain chlorophyll-corpuscles.

b. The **phloem,** recognized as a tissue with thin cellulose walls, small cavities, and sparing protoplasmic contents: though reduced in bulk at the poles of the

elliptical bundle, it forms a continuous band surrounding—

c. The central **xylem,** which appears as a spindle-shaped mass of tissue when seen in transverse section, and consists of elements with lignified walls, and no cell-contents.

Small vascular bundles of rounded outline, as seen in the transverse section, may be found opposite or near to the ends of the spindle-like vascular bundle; these are bundles of the leaf-trace cut through on their course inwards from the leaves: thus the whole bundle-system of this shoot consists of a single central bundle, which traverses the axis longitudinally, and gives off smaller branch-bundles, which pass outwards into the leaves, one of them entering each leaf.

The vascular system of the shoot is more complicated in certain other species of *Selaginella*, and transverse sections should be made from these: thus in *S. Willdonovii*, *S. inæqualifolia*, &c., three large flattened bundles are seen in each transverse section of the axis; the planes in which these bundles are flattened are parallel to one another; in these species the bundles may individually show considerable irregularities of outline: the whole arrangement of their vascular system may with advantage be compared with that in the stem of *Lycopodium*.

Note with a higher power—

1. The general appearance of the **phloem,** with its highly refractive cellulose walls, and scanty protoplasm.

2. Between this and the xylem a somewhat irregular series of cells of the **conjunctive parenchyma,** with thin cellulose walls and plentiful protoplasm.

3. The chief constituents of the **xylem** are large

prismatic **tracheides,** with peculiarly marked, lignified walls.

4. At the poles of the spindle-shaped xylem note tracheides of smaller size: these compose the first-formed **protoxylem:** the development of the xylem thus starts from the periphery and proceeds towards the centre of the oval xylem.

To confirm this, cut transverse sections of the stem about one inch from the apex, and treat as above. It will be seen that the elements near the poles of the xylem are already fully formed, and their walls lignified, while those at the centre are still thin-walled, and have protoplasmic contents. The lacunar tissue will be found to be better defined in these sections.

IV. Cut longitudinal sections of a stem of *S. Martensii.* Owing to its being fixed in its place only by the weak trabeculæ, the vascular bundle is apt to be detached in cutting accurately longitudinal sections: it will therefore be found better to cut the sections slightly oblique; it must then be remembered in examining them under the microscope that the sections are not truly longitudinal.

Mount some in glycerine, others in chlor-zinc-iodine, and examine them under a high power, noting the same succession of tissues on starting from the outside as were seen in the transverse sections: thus—

1. **Epidermis** } these are hardly to be distin-
2. **Outer cortex** } guished one from another: the cells of both are prosenchymatous, and thick-walled, and show a gradual transition to—

3. The **inner cortex,** in which the walls are thinner, and the form of the cells parenchymatous.

4. The **lacunar tissue,** in which the outer parenchyma, consisting of short and small cells, is to be distinguished from the inner cells, which are elongated in a radial direction, and show that peculiar median constriction above noted in the transverse sections.

5. The **pericycle,** consisting of elongated parenchymatous cells, with cellulose walls, and often containing chlorophyll.

6. The **phloem,** the most prominent elements of which are long narrow elements with cellulose walls and sparing contents: these are regarded as the representatives of the **sieve-tubes.**

7. The **xylem,** the most prominent elements of which are **spiral** and **scalariform tracheides,** similar to those to be described below as composing the xylem of the bundle in *Lycopodium* and in the Ferns. The walls are lignified and thickened, and marked by elongated pits, which by their regular arrangement give the scalariform character to these elements.

V. Cut transverse sections of a rhizophore: mount as before, and observe that the peripheral tissues are not unlike those of the stem, and are marked off from the central cylinder by a somewhat irregular **bundle-sheath** or **endodermis;** that the arrangement of tissues of the central cylinder differs both from that of the stem, and that usual for root-structures, there being but one group of **protoxylem** (monarch), which is placed laterally: the later-formed **xylem** forms together with it a central mass, which is surrounded by **phloem** except at the point opposite the protoxylem. The structure of the individual vascular elements is similar to that in the stem.

If successful median longitudinal sections be cut through the apex of a rhizophore, it will be found that there is no root-cap. Further, by comparison of a number of sections, both longitudinal and transverse, it may be concluded that there is one **apical cell** having approximately the form of a quadrangular prism or pyramid.

VI. Cut transverse sections of a root, and mount as before: the structure will be found to resemble that of the rhizophore, as above described.

If median longitudinal sections be cut through the apex of a root, a **root-cap** will be seen, which covers an **apical cell,** having the form of a triangular pyramid. Observations may also be made on the endogenous formation of the roots at the apex of the rhizophore, when the latter reaches the surface of the soil.

VII. Mount some leaves, both dorsal and ventral, which have been previously bleached in alcohol, in water, or dilute glycerine: examine with a low power, and observe the difference in form of the dorsal and ventral leaves, their ciliate margin, and central midrib.

Note carefully the character of the **epidermis**: thus on the **upper surface** the cells are small, and circular in surface view, with sinuous lateral walls, and **no stomata**; the cells of the **lower surface** are elongated, with pointed ends, and sinuous lateral walls; over the midrib the cells are shorter, and it is there only that the **stomata** are found, having two guard-cells, and no subsidiary cells.

VIII. Cut transverse sections of fresh leaves held in a piece of pith: mount in water or weak glycerine, and observe—

1. The **epidermis** of the upper surface consists of

conical cells, each of which contains a single large **chlorophyll corpuscle,** which is usually closely applied to the internal wall of the cell: in sections suitably stained it may be seen that the nucleus of the cell is closely applied to the large chloroplast. Stomata are absent.

2. Beneath this is the **spongy parenchyma,** which incloses centrally—

3. A single **vascular bundle.**

4. The **epidermis** of the lower surface consists of smaller cells containing chlorophyll, and with **stomata,** opposite the midrib: note the two small guard-cells as seen in transverse section. Near the margin of the leaf the upper and lower epidermal layers are in contact with one another, the spongy parenchyma being there absent. There is also a marginal band of thickened cells.

IX. Choose out from material which has been hardened in alcohol the **apical buds** of branches which have not as yet begun to form sporangia: holding these between pieces of pith or carrot, or, better, embedding them in paraffin, cut longitudinal sections: mount them in glycerine, examine first with a low power, and select those sections which are nearest to the median plane, *i.e.* those which show the greatest regularity of parts, and the stem terminated by the apical cone.

In such sections observe—

1. The **axis,** with tissues as above described in the longitudinal sections, and terminated by the apical cone: borne laterally on this are—

2. The **leaves,** each having a **ligule** attached to its upper surface: note also their structure as above

described. Passing towards the apex of the bud observe successively earlier stages of their development.

Examine the sections with a higher power, and observe—

1. The arrangement of the cells at the summit of the apical cone, which is terminated by a single conical **apical cell**: from this segmental cells are successively cut off.

2. The origin of the leaves is not from a single cell, but by the outgrowth and subsequent division of a number of cells at the periphery of the apical cone.

3. Occasionally examples of **branching of the axis** will be found, and the lateral branches originate below the actual apex, though above the youngest leaves, by bulging out of a mass of cells: the branching is thus monopodial.

In such median longitudinal sections the differentiation of the vascular bundle from the primary meristem should also be observed, and also the development of the lacunar tissue, and its relation to the central bundle.

Preparations may also be made of the apex so as to show the structure of the apical cone as seen from above. By comparison of a number of these it may be seen that the form of the apical cell is by no means constant, but varies between the forms of a two-sided and a three-sided cone. If the sections be not sufficiently transparent, they may be treated with "eau de javelle" before mounting in glycerine.

X. Cut longitudinal sections through fertile branches similar to those cut from the vegetative bud, and examine them under a low power.

Observe that the general arrangement of the stem, leaves, and ligules is the same as in the vegetative

bud. In the lower part of the sections a mature **sporangium** may be found in the axil of each leaf. The sporangium may have lost its spores partially or entirely during the preparation of the sections. It will consist of—

a. A short massive **stalk.**

b. A **wall** inclosing the central cavity: the wall will be found under a high power to consist of three layers of cells—

i. The outer consisting of thick-walled cells, more or less elongated radially.

ii. A layer of small, compressed cells.

iii. A layer of thin-walled cells, elongated radially: this is the **tapetum,** which is here persistent until the spores are ripe.

Surrounded by the wall will be found—

c. **Spores** of two sorts, contained in different sporangia—

i. **Microspores** of relatively small size: these will be found in large numbers in certain sporangia, which will accordingly be recognized as **microsporangia.** When ripe they may be still seen to cohere in groups of four: each spore is a single cell with a brown wall.

ii. **Macrospores** of relatively large size: **four** only of these will be found inclosed in a single sporangium, which is accordingly termed a **macrosporangium.** Each spore consists of a thick wall, with numerous external projections, surrounding a large cavity filled with protoplasm, &c.

The development of the sporangium may be traced in longitudinal sections of sporangium-forming cones which have been hardened in alcohol, or in picric acid and then in alcohol: mount

in glycerine. The following points in the process of development may be observed :—The sporangium is first seen as a swelling of a group of cells at the surface of the apical cone, above the leaf in the axil of which it appears : thus the sporangium is not borne on the leaf as in *Lycopodium*, but springs from the tissues of the axis. A central row of cells grows more strongly than the rest, and the outermost cell but one of this series may be recognized as the **archesporium.** The outermost cell divides to form part of the two outer layers of the wall of the sporangium. The archesporium also divides to form a mass of tissue, of which the peripheral layer becomes the **tapetum** (the basal part of the tapetum is however derived from the adjoining tissue). The central part of the tissue derived from the archesporium forms the spores : each spore-mother-cell separates from its neighbours, and divides into **four** cells. If the sporangium is to develop **macrospores,** only one of these groups of four cells is further developed, the rest being abortive; if it form **microspores,** all the groups of four are further developed, but only attain a comparatively small size : in both cases the four spores may separate from one another when quite mature, though they often retain their original arrangement.

THE GAMETOPHYTE OR OOPHYTE.

XI. Spores of both kinds may be obtained free by drying branches which bear sporangia on sheets of paper. Pick out the **macrospores,** and mount them in olive-oil : dissect off the brittle outer coat of the spore with needles, and examine under a high power. It will be seen that the chief contents of the ripe spore are a protoplasmic matrix inclosing oil-globules and aleurone-grains, while traces of the cells of the **prothallium** may be recognized even in these preparations.

Prepare other such spores with potash, and dissect as before, or press on the cover-slip, and warm gently. It will be found, when the oil, &c., has been acted upon

by the potash, that a part of the contents of the spore is traversed by a distinct network of cell-walls, forming a meniscus-shaped mass of tissue. If plenty of spores are to be had, it will be found better to embed a quantity of them, and to cut sections, mounting them in glycerine. Observe—

1. The character of the wall, consisting of—

a. An outer thick, yellow **exospore.**

b. An inner thin **endospore.**

2. The contents as above described: the natural position of the cellular tissue of the **prothallium** may be seen to be at the apex of the cavity of the spore.

For the more accurate study of the contents of the macrospore, serial sections should be cut with the microtome, stained, and mounted in canada balsam: it is by this means that the details of the prothallus can be observed with greater ease and certainty.

XII. Spores of both kinds should be collected in considerable quantity by drying on paper, and then be sown on moist soil or sand, and left to germinate. In a few weeks young seedlings will be seen with an erect axis, bearing small leaves. Note that the axis of the seedling branches at an early period.

Remove some of these seedlings from the soil, and note the monopodial branching of the root, and the macrospore still attached laterally to the axis.

Longitudinal sections should be made through the young seedling, so as to traverse also the macrospore attached to it: in such sections it will be readily seen that a lateral outgrowth (the **foot**) projects from the base of the axis into the cavity of the macrospore: also that the latter is filled with a cellular tissue of the

prothallus, from which the nutritive substances above noted in the mature macrospore will have been removed.

A careful microscopic comparison of the spores sown as above directed should be made from time to time during the process of germination: it may then be ascertained that the contents of the microspores divide into a number of cells, and ultimately rupture and set free **spermatozoids**: also that the tissue of the **prothallus** in the macrospore increases, rupturing the wall of the spore: that **archegonia** are formed, from one of which the young seedling originates. For further details the text-books must be consulted.

II. Homosporous Type

LYCOPODIUM CLAVATUM (The Common Club-Moss)

SPOROPHYTE

I. In a well-grown specimen of this plant, which is commonly to be found on moorland hills, recognize the following external characters:—

1. The **stem,** often extended to a great length, is creeping, and frequently branched, apparently in a monopodial manner (for particulars see p. 283): the stronger branches are also creeping, the weaker branches ascending.

2. From the under side of the stem **roots** are developed, which frequently, but not always, appear at points where the stem branches. The roots themselves are branched dichotomously, but the limbs of the dichotomy may develop either equally or unequally.

3. The stem is covered with **leaves,** which are simple in form, and linear, with ciliate margin, and have a long awn-like apex. The arrangement of the leaves is complicated, and has been described as being partly in whorls, partly spiral: the number of members of the whorls is variable, as is also the angle of divergence of the spirally-arranged leaves.

4. The fertile branches or **cones,** which bear

sporangia, are erect and elongated; their lower part is covered sparsely with leaves of small size: about 1–2 inches below the apex they usually divide into two or three branches, covered with rather broader, closely imbricated leaves: from the upper surface of each of these rises one **sporangium,** which is yellow when ripe, and opens by a split parallel to the plane of the leaf which bears it.

II. Cut transverse sections of a fully developed stem: mount some of them in glycerine, others in chlor-zinc-iodine, and examine with a low power: externally will be found—

1. An **epidermis,** consisting of a single layer of cells: their outer walls are thick, and covered by a continuous layer of **cuticle,** which may be recognized in thin sections by its high refractive power, and yellow stain in chlor-zinc-iodine.

2. Below the epidermis lies a broad band of **cortical tissue,** which appears differentiated into successive thinner bands according to the thickness of the cell-walls: thus there may be distinguished—

a. An external **sclerenchymatous** band, with thick lignified walls (brown with chlor-zinc-iodine) and scanty protoplasm: small intercellular spaces may be seen at the angles between the cells.

b. Within this is a broad band of **thin-walled tissue,** in which the cell-contents are not apparent: the cell-walls are tinged with pink in chlor-zinc-iodine. There is a gradual transition from this to—

c. The most central part of the cortex, which is strongly **sclerenchymatous:** it has intercellular spaces, and retains its cell-contents. It forms a dense

band of lignified tissue (brown with chlor-zinc-iodine). Here and there may be found in the cortex small groups of very narrow elements, having a dark appearance: these are single **vascular bundles of the leaf-trace,** cut through on their course from the leaves to the central vascular cylinder. A sudden transition is found from the inner sclerenchymatous band (*c*) of the cortex to—

3. The bundle-sheath or **endodermis,** which lies next to it internally. This tissue consists of two to three layers of tangentially elongated cells, the walls of which have a sharp contour, are not thick, and stain with chlor-zinc-iodine a slightly different tint of brown from the walls of the sclerenchyma: this band, though it consists of more than a single layer of cells, is regarded as taking the place of the endodermis, which is met with in most of the Pteridophyta as one definite layer of cells.

Treat a section with sulphuric acid: the walls of this band, being of a corky nature, will be found to resist its action more than the rest of the tissues. Note also that the cuticle is brought into greater prominence by treatment with sulphuric acid, since it resists the action more strongly than the other walls.

Within this endodermis is found—

4. A cylindrical mass of **vascular-tissue:** it is composed, as in other cases, of (*a*) **phloem-tissues,** and (*b*) **xylem-tissues,** which may be distinguished by their optical properties, and by their mode of staining with various reagents. Observe that the phloem forms a series of bands which alternate more or less regularly with the masses of xylem; the latter are of

elliptical form as seen in the transverse section: several of these are ranged side by side, the longer axes of the ellipses being horizontal as the plant grows, and parallel to one another: the masses of xylem may be irregularly connected one with another towards the centre of the stem.

III. Before proceeding to the study of these several tissues in detail, cut transverse sections from the young stem, at about one-eighth of an inch below the apex: treat some of these with dilute potash or "eau de javelle," others with chlor-zinc-iodine. Observe that in these sections the tissues at the centre of the vascular cylinder are still thin-walled, and have plentiful protoplasm, *i.e.* they are not fully developed; towards the periphery, however, will be seen a series of groups of tissue showing the characters of developed xylem, and alternating with these is a series of groups of phloem tissues. Other preparations may be made successively from points further from the apex, and from these the conclusion may be drawn that the vascular tissues at the periphery of the vascular cylinder are matured first, and that the development proceeds towards the centre.

IV. Returning to the sections of the old and mature stem, examine the vascular tissues under a high power.

i. Immediately within the endodermis is a band of tissue, which abuts directly upon the periphery of the xylem and phloem, and having cell-walls which stain blue with chlor-zinc-iodine: this may be regarded as the phloem-sheath or **pericycle.**

ii. On examining the masses of **xylem,** observe that—

a. The constituent elements are much smaller at the periphery of the vascular cylinder than towards the centre: the former are the first developed or **proto-xylem** elements.

b. The main constituents of the xylem are elements with large cavity, and of rounded polygonal form (**tracheides**) : note the structure of the wall, especially where two of these adjoin one another.

iii. In the **phloem** there will be found at the periphery of the vascular cylinder, and alternating between the successive groups of protoxylem, masses of tissue with thick cellulose walls, and small cell-cavities : these are the **protophloem** groups, or first-formed elements of the phloem. Passing from these towards the centre of the vascular cylinder, the phloem is found to consist of—

a. Constituents with large cavities, and very scanty cell-contents.

b. Elements with small cavity, and obvious cell-contents.

V. Cut radial longitudinal sections through a mature stem : mount as before, examine them first with a low power and note—

1. The bases of the leaves continuous with the epidermis and cortex of the stem.

2. The **cortex** showing the same differentiation into successive bands (*a*), (*b*), (*c*), as was seen in the transverse section.

3. The central **vascular cylinder.**

4. Small **vascular bundles of the leaf-trace,** which may be seen pursuing an oblique downward course from the bases of the leaves, through the cortex, to the periphery of the vascular cylinder : since these bundles may be followed in one radial section from the leaf to the central cylinder, it follows that that part of their course is approximately in a radial plane.

We can now obtain a clear idea of the **vascular system** of a mature shoot of *Lycopodium clavatum* there is in the first place a central vascular cylinder, which traverses the shoot longitudinally, and from the periphery of it single bundles of small size are given off, which take an obliquely ascending course in radial planes, and each of them enters a leaf. By further comparison of longitudinal and transverse sections, it may be ascertained that the masses of xylem in the central cylinder have the form of flattened plates, the planes of which are approximately horizontal in the living plant. They are sometimes separate from one another, sometimes joined towards the centre of the stem, and it is on the margins of these plates that the bundles of the leaf-trace are inserted.

Examine the radial longitudinal sections under a high power, and observe—

i. That the cells of the epidermis, and the sclerenchymatous portion of the cortex, are elongated and **prosenchymatous,** while those of the thin-walled band of the cortex are shorter, and tend to a **parenchymatous** form. The walls of the cortical cells are pitted.

ii. Of the vascular tissues the **xylem** is the most prominent; its chief constituents are of prosenchymatous form, with lignified walls: the latter show the **scalariform** marking which is due to the regular arrangement of elongated pits with their longer axes placed horizontally: each of these pits shows a double contour, and transitional forms will be found from the elongated to circular pits, the latter presenting an appearance very similar to that of the bordered pits

of *Pinus* (p. 240), from which these elongated pits differ only in their outline as seen in surface view. Some of the walls will have been cut through longitudinally: examine a section of one of them, and note especially that the **pit-membrane** is constantly present; there is thus no direct communication between the cavities of these elements, and they have no cell-contents remaining; they are therefore **scalariform tracheides**: compare those in the xylem of the Fern, and the tracheides of *Pinus* (p. 238).

iii. Where the sections have passed through the peripheral margins of the plates of xylem, there will be found elements of the **protoxylem,** which correspond in structure to those in the stem of *Pinus:* irregular finger-like outgrowths of the cell-wall may be observed extending into the cavity of these elements.

iv. The phloem, intervening between the masses of xylem consists of—

a. Prosenchymatous cells with cellulose walls, and granular cell-contents: these are directly in contact with the xylem.

b. Long tubular structures, the pointed endings of which are very rarely met with: their course may be followed for a considerable distance in the longitudinal direction: they have transparent contents, and their cellulose walls are dotted with minute pits, about which bright globules adhere. These are probably the representatives of the **sieve-tubes** of other vascular plants.

Stems of other species of *Lycopodium* may be treated in the same way, and a comparison made of their structure: the general arrangement of tissues will be found to be fundamentally the

same as that described for *Lycopodium clavatum*, the differences depending chiefly upon the number of plates of xylem and phloem, and variations in the manner and extent of the connection between the plates of xylem.

VI. Cut transverse sections of the leaf: this may easily be done either by embedding the whole stem, with the leaves attached, in paraffin, cutting transverse sections of the whole, and then picking out the sections of the leaves; or by holding the stem with the leaves between the finger and thumb, and cutting transverse sections from the whole as from a solid mass. Mount as before: examine under a low power and note—

1. The outline of the sections roughly triangular.

2. The single layer of **epidermis** with cuticularized outer wall: **stomata** are found on both the lower and the upper surface.

3. Beneath this is the **mesophyll,** with large intercellular spaces: the cells, which form an irregular network, are nearly globular, have thin walls, and contain chlorophyll-corpuscles.

4. At the centre is a single very small **vascular bundle.**

VII. Cut median longitudinal sections through the bud: use material which has been preserved in alcohol, or hardened in picric acid and then in alcohol. Mount in glycerine, and examine with a low power: note that at the lower part of the section the central vascular cylinder will be easily recognized, while the bundles from successive leaves pass obliquely through the cortex, and insert themselves upon its margin. Passing upwards, however, towards the apex, it gradually loses its dark appearance (due to developed xylem); still

its continuity may be traced up to the apical cone, as a bright-looking strand of formative tissue consisting chiefly of prosenchymatous elements (**plerome** or **procambium**), while the formative tissue of the cortex external to it is more typically parenchymatous (**periblem**): it is limited by a layer of cells, which, though not clearly defined towards the extreme apex, may be recognized as the **dermatogen.**

The conical apex itself consists of a dome-shaped mass of meristem: the layer of dermatogen, which may be recognized at the base of the cone, may be followed up nearly to the apex, but there loses its identity, the extreme apex being occupied, at least in the more bulky examples, by a group of **initial cells** (stated by Strasburger to be three in *L. Selago*), which divide by both anticlinal and periclinal walls. Compare this on the one hand with the structure of the apex of the stem of Phanerogams (p. 80), and on the other with that seen in the Ferns.

Observe further that the **development of the leaves** begins by the outgrowth and division (both anticlinal and periclinal) of groups of cells, which constitute multicellular protuberances: these have at first an apical growth, which soon ceases, the further growth being basal and intercalary.

The origin of the **branches** may further be observed in these preparations: it will be seen that they arise in this species below the apex of the main axis, and laterally upon it; the branching is thus **monopodial,** not dichotomous.

VIII. Cut transverse sections of one of the thick roots: mount as before, and observe that they re-

semble the transverse sections of the stem in the arrangement of the tissues, though the whole structure is simpler: there are usually only three plates of xylem: these are often complicated by irregularities, *e.g.* fusion, &c., and are less strongly developed in their central portion than those of the stem.

If similar sections be cut successively from roots of higher order, they will be found to show successively reduced types of structure, till the xylem is finally represented only by a single group of elements, which is surrounded by tissues of the phloem.

Median longitudinal sections may be cut through the apices of roots which have been hardened in alcohol: from these it will be seen that there is a stratified structure of the apical meristem, in which may be recognized a distinct **root-cap,** marked off from the layers below it by a layer of **dermatogen,** which can be traced as a continuous layer beneath the root-cap: centrally a distinct strand of **plerome** may be recognized. Bifurcations may be found in such sections, showing that the branching is a true **dichotomy.**

IX. Cut median longitudinal sections through a cone, bearing **mature sporangia:** mount in glycerine, and examine under a low power: observe the structure of the axis as before seen in longitudinal section, with a vascular system sending out branches into the leaves: the chief difference between this and the vegetative axes is the presence of **sporangia.** Note—

i. That one sporangium is seated with a short stalk on the upper surface of each leaf.

ii. That no branch of the vascular system enters the stalk of the sporangium.

iii. That the cavity of the sporangium is surrounded by a thin wall.

iv. That the cavity thus inclosed may be filled with

small tetrahedral spores: in preparing the sections, however, the spores are often washed out from the sporangia.

Examine a good section of a sporangium under a high power: the wall will then be seen to be of approximately uniform thickness throughout, and consists of—

a. A well-marked outer layer of cells of considerable size.

b. An inner ill-defined band, consisting of the remains of disorganized cells.

If the wall be observed in surface view, the cells will be seen to be of sinuous outline, and somewhat elongated, with the exception of a zone which indicates the line of ultimate dehiscence of the sporangium: here the cells are shorter, and the walls are straight. The line of dehiscence may also be recognized as a thinner point in the wall as seen in section. Note also the structure of the **spores**; they have the form of a rounded tetrahedron, and the outer wall is covered with peg-like projections.

By cutting similar median longitudinal sections of cones in various stages of development, and comparing them, the history of development of the sporangium may be traced. It may be seen that the sporangium arises as a multicellular outgrowth of the upper surface of the leaf: at an early stage the **archesporium** may be recognized in these sections as a hypodermal cell, or even several cells: a careful comparison of tangential or transverse sections shows that there is a row of five to eight archesporial cells, of which usually one only appears in a thin radial section: the superficial layer of cells above it gives rise by division to three layers; of these the innermost is the **tapetum**, which, together with the next outer layer, is disorganized as development proceeds, while the outermost layer is still persistent in the mature

sporangium. The archesporium meanwhile divides to form numerous **spore-mother-cells,** each of which divides tetrahedrally, and gives rise to four spores.

Though in recent years great additions have been made to the knowledge of the prothallus in various species of *Lycopodium*, still the conditions favourable for the germination of the spores are not sufficiently well known to make it probable that the attempt to grow the prothalli would be successful: accordingly reference should be made to the text-books, and to special memoirs for further information as to the characters of the prothallus.

B.—FILICINEÆ

I. Homosporous Type

ASPIDIUM FILIX-MAS (The Male Shield Fern)

A.—MATURE SPOROPHYTE

I.—*External Characters*

I. Taking a well-grown plant of the common Male Fern in summer, wash the soil away from the roots, and observe the following external characters :—

A. The **stem** is oblique and ascending: it is not branched at its apex: its surface is covered by the persistent bases of the **leaves,** which are densely covered by numerous brown scaly hairs (**paleæ** or **ramenta**).

B. The **leaves,** the most prominent of which are—

i. The fully-developed green leaves of the current year : these are large and of complicated structure and the following parts may be recognized :—

a. A long almost cylindrical leaf-stalk, which is traversed by two longitudinal, lateral ridges or reduced wings. This leaf-stalk supports—

b. The numerous **pinnæ,** which are arranged in two lateral rows, corresponding in position to the lateral ridges above mentioned : note that the arrangement of the nerves in the segments of the pinnæ is based upon

repeated bifurcation of the stronger nerves. On the under side of the pinnæ will frequently be found—

c. **Sori,** which are roundish brown groups of small stalked bodies (**sporangia**), covered by a kidney-shaped **indusium.**

ii. The bases of the leaves of previous years will be seen, covering the lower part of the stock or stem externally: observe that lateral **buds** are frequently to be found connected with these, being attached to their ab-axial side, near to their point of junction with the stem.

iii. Nearer the apex of the stem than the expanded leaves of the current year, and completely covering it, are **young leaves,** densely covered with ramenta: these, together with the axis, constitute the **apical bud.** Note that the apex of each such leaf is rolled up like a crozier (**circinate vernation**).

Here, as in most Ferns, the development of the leaves is very slow: the young leaves seated round the apex represent the foliage-leaves of the two succeeding years.

C. The **roots** are rather thin and brown, with transparent apices: they are inserted **on the bases of the leaves,** close to their junction with the stem: the branching of the roots is monopodial, and their branches appear in acropetal succession.

The stem of *Aspidium Filix-mas* does not branch at its apex: the same is normally the case in the erect stems of Ferns (*e.g.* Tree-Ferns) where the leaves are closely crowded. In those Ferns in which the axis is elongated, a terminal branching is more frequent: thus in *Pteris aquilina* there is occasionally a dichotomous branching. In other forms the new axes appear in connection with the

leaves, either at the base of the leaf (*Aspidium Filix-mas*), or in various positions on the flattened upper part of the leaf (many species of *Asplenium*).

II.—*Anatomical Characters to be observed with the naked eye.*

II. Having observed the above external characters, remove the roots, keeping the transparent apices of the young roots, as well as the thickest parts of the old roots: these should be preserved in alcohol for further treatment.

Starting from the older end of the stock, cut off successively the persistent bases of the old leaves about half an inch above their insertion on the stem. Observe the lateral bud borne by some of the leaves on the ab-axial side of the leaf-stalk near its base: observe also that the roots spring from the bases of the leaves, close to their insertion on the stem.

Cut off about 2 inches of the older end of the stem exposed as above, and boil it in dilute hydrochloric acid till the parenchyma is soft: for further treatment of this see below. Meanwhile smooth the cut end of the remainder of the stock with a razor, so that it may present an even surface of transverse section, and observe—

a. The great irregularity of outline, due to the close crowding of the bases of the leaves.

b. The dark brown band of **sclerenchyma** bordering the periphery of the section.

c. The great bulk of the stem consisting of yellowish **parenchyma,** with very bulky central **pith.**

d. Round the latter are a number of isolated, **large vascular bundles**, forming an interrupted ring.

e. Outside these, and running out into the leaves, are numerous smaller **bundles of the leaf-trace,** which appear to be less regularly arranged.

III. Divide the stock, including the apical bud, into two symmetrical halves by cutting it in a median longitudinal plane: smooth one of the cut surfaces with a razor, and observe—

a. That the stem is of almost equal thickness throughout its length, *i.e.* it is roughly cylindrical.

b. That its external conformation is very irregular by reason of the closely crowded insertion of the leaves.

c. The bulky central pith as before.

d. The large vascular bundles (*d* above), which are not continuous in direct longitudinal lines, but form an interrupted series.

e. The smaller bundles of the **leaf-trace** (*e* above), which in some cases may be followed, after a little careful dissection of the parenchyma which surrounds them, from one of the larger bundles of the central system into the base of one of the leaves.

Slice away carefully the external tissues of the posterior part of the stock, so as to lay bare the central system of larger bundles: it will then be seen that these form a continuous **network** with large meshes, and that each mesh is opposite the point of insertion of one of the leaves, hence it is called a **foliar gap.** Observe also that the vascular bundles, which pass out into any individual leaf, are given off from the margin of its own mesh.

IV. Confirm these observations by the dissection of the part of the stock macerated in dilute hydrochloric acid. The parenchyma, being thus rendered soft and friable, may be easily removed, leaving the vascular system as a network of stronger bundles, which gives off numerous weaker bundles from the margins of its meshes: these weaker bundles run out into the leaves.

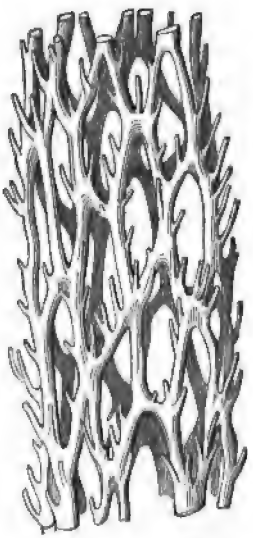

FIG. 12.—Vascular skeleton prepared by maceration and dissection from the stem of *Aspidium Filix-mas*. (After Reinke.) (2 : 1.)

By careful dissection a skeleton may be prepared similar to that shown in Fig. 12.

Though the above is the general type of bundle-arrangement for Ferns with ascending or upright stems, in Ferns with creeping stems other modes of arrangement are found, which, however may be regarded as being related to the type above described.

Thus (1) in the *Hymenophyllaceæ*, &c., there is a single central bundle, an arrangement which is found also in the young seedlings of other more complex forms; (2) in species of *Davallia* and others with horizontal stems the ring consists of two stronger bundles, one running parallel to the upper, the other to the lower surface; between these are on each side several smaller bundles which, together with the two stronger ones, form an interrupted ring as seen in transverse section; (3) in other cases there are several (in *Pteris* two) concentric rings of bundles, which give off branches to the leaves, &c; (4) again, in the stems of Tree-Ferns a cylindrical network is present, somewhat like that of *Aspidium*, but with additional bundles which ramify in the central pith, and also in some cases in the cortex.

There is great variety also in the disposition of the strengthening tissue or sclerenchyma in different types of Ferns: thus it may be absent, as in species of *Davallia;* or it may be present as a thin peripheral band, as in *Aspidium;* or it may be present as thick brown masses, as in *Pteris* and in Tree-Ferns.

V. Remove from the apical bud the large quantities of scaly hairs (ramenta), so as to lay bare—

1. The **young leaves,** with their circinate vernation.

2. The broad **apex of the stem** with leaves in various stages of development around it.

3. The **young roots** which will be found already present on the bases of very young leaves.

4. The **young buds** which may be observed at a very early stage on the posterior side of the leaves.

III.—*Microscopic Investigation.*

VI. Cut transverse sections of the stock of *Aspidium:* it is hardly to be expected that a transverse section of so bulky a stem as this could be cut so uniformly thin that the structure of all the tissues could be well seen;

it is better therefore to cut a number of sections, each extending over a comparatively small area, and to study the various tissues separately. Mount some in glycerine or glycerine jelly, others in chlor-zinc-iodine. Examine under a low power, and observe successively the following tissues, starting from the periphery of the stem :—

a. An **epidermis,** consisting of a single, somewhat irregular and ill-defined layer of cells, with dark brown outer walls: their arrangement is disturbed at the point of insertion of the **scaly hairs** which appear as plates of cells, one layer of cells in thickness, rising obliquely from the epidermis. Beneath this is—

b. The **ground-tissue,** which is differentiated as—

i. An outer narrow band of tissue, with rather thick, colourless, pitted walls, and cell-contents with much starch: there are no intercellular spaces.

ii. A band of **sclerenchyma** with thick, yellow, lignified, obviously stratified, and pitted walls, cell-contents as in (i.), and no intercellular spaces. This merges gradually into—

iii. The bulky central mass of ground-tissue, in which the vascular bundles are embedded. It consists of cells with comparatively thin, pitted, cellulose walls, protoplasmic contents with much starch, and with intercellular spaces.

On the external surface of those parts of the cell-walls which adjoin the intercellular spaces numerous small projecting spikes may be observed: it may be readily seen that these originate in connection with the formation of the intercellular spaces.

Internal glandular hairs are also found in the intercellular spaces: they are attached by narrow stalks to single cells of the

parenchyma: the globular head contains when fresh a resinous secretion, which is soluble, but not readily, in alcohol.

c. The **vascular bundles** of elliptical outline: they are embedded in the ground-tissue, and are sharply circumscribed by a narrow, light brown layer of cells without intercellular spaces: this is the **bundle-sheath** or **endodermis.** Among the tissues inclosed by this sheath, note that a large central mass may be distinguished as consisting for the most part of elements with large cavity, no cell-contents, and rather thick walls with a peculiar marking: this is the **xylem.** Between this and the bundle-sheath is a broad band of tissue with thin, bright looking walls, and with protoplasmic contents: this is the **phloem.** Since the xylem is surrounded by the phloem, this bundle is said to be of the **concentric type.**

In the sections treated with chlor-zinc-iodine note that the walls of the inner ground-tissue stain blue, and that starch is found in the cells; that the bundle-sheath appears browner than before; that the walls of the phloem stain blue (cellulose), and the contents yellowish; that the walls of the chief constituents of the xylem stain yellow (lignified).

VII. As the vascular bundles of the leaf-stalk are better fitted for minute observation, and are better types of the concentric bundle of the Fern than those of the stem, cut thin transverse sections of the lower part of the petiole. Having previously noted with a low power that in their main features the tissues resemble those above observed in the stem, examine the structure of one vascular bundle under a high

power, and starting from the periphery of it, note successively—

1. The **bundle-sheath** or **endodermis,** a single layer of cells, with yellowish walls, and yellow granular contents: there are no intercellular spaces in this layer, nor in any of the tissue surrounded by it.

Treat a thin section with sulphuric acid, and note that the walls of the endodermis retain a sharp contour, while those of the rest of the tissue swell, and become more or less disorganized.

2. The **phloem-sheath** or **pericycle,** which is a band of tissue of varying thickness at different parts of the bundle, being thin at the poles of the elliptical bundle, and thicker at the sides: it consists of cells of roundish form, with cellulose walls, and protoplasmic contents, and starch.

3. At the inner limit of the pericycle are found elements with thick cellulose walls and narrow cavity, these constitute the **protophloem.**

4. Internally lies the broad band of true **phloem,** which is composed of two tissue-forms—

a. **Sieve-tubes,** which appear polygonal in the transverse section, with thin cellulose walls which are lined by a delicate protoplasmic membrane dotted with numerous highly refractive granules.

b. Cells of the **conjunctive parenchyma,** with thin walls, and protoplasmic contents.

5. Centrally lies the **xylem,** in which also two constituents may be recognized—

a. **Tracheides,** which appear polygonal in section, and have large cavities, with no cell-contents: the walls are thick and lignified, and show a

peculiar structure which will be better understood on comparison of their appearance in longitudinal sections.

b. **Conjunctive parenchyma** with cellulose walls, and protoplasmic contents with starch: these cells are distributed evenly throughout the xylem, and also form a band surrounding it completely.

VIII. As a preliminary to the study of longitudinal sections, separate some pieces of the vascular bundles of *Pteris* or *Aspidium* from the surrounding tissue, and warm them gently in a test-tube with a little potassium chlorate and nitric acid, till the elements of the bundle may be separated easily one from another; then stop the action by diluting with water, and mount in water or glycerine. By preparing them in this way the tracheides, &c., may be subjected to separate examination, and their form and structure may be more exactly made out.

Apply the same process to the sclerenchyma, and observe the form and marking of the walls of its constituent elements.

Cut longitudinal sections of the stem of the Male Fern: first take radial sections of the peripheral tissues, and treat as above: note—

1. The **epidermis** with scaly out-growths.

2. The subjacent ground tissue, and especially the **sclerenchyma** consisting of cells of short prosenchymatous form, with brown pitted walls, and cell-contents: note the gradual transition from sclerenchyma to—

3. The colourless ground tissue, with short parenchymatous cells, and large intercellular spaces.

IX. Cut longitudinal sections so as to pass tangentially through the central network of bundles: treat some sections with chlor-zinc-iodine, and mount others in glycerine. Note the several tissues observed in the transverse sections, and they will show here the same position relatively to one another. By reason of the frequent splittings and fusions of this bundle-system the several elements will appear contorted and twisted, but this does not materially affect their general arrangement, which will be seen under a high power as follows:—

A. In the **xylem** of the bundle—

a. The **scalariform tracheides,** which are the main constituents of the xylem: they are elongated, prosenchymatous elements, with ladder-like marking of the lateral walls: this is due to the presence of regularly arranged, transversely elongated, **bordered pits.** (Compare those of the pine, p. 240.) Take especial notice of the appearance of the lateral walls as seen in longitudinal section, where two tracheides are contiguous with one another, and compare them with parts of the wall which adjoin.

b. Cells of the **conjunctive parenchyma** interspersed among the tracheides.

c. Tracheides with spiral marking: these are the first-formed wood, or **protoxylem.**

B. In the phloem observe—

a. The **sieve-tubes,** which are also elongated elements with pointed ends; the surfaces of the walls which separate contiguous sieve-tubes are covered with

numerous **sieve-plates** (best seen in sections treated with chlor-zinc-iodine), to which round, highly refractive granules adhere: these stain yellow with chlor-zinc-iodine. Note also the irregular beaded appearance of the walls when seen in longitudinal section.

b. Cells of the **conjunctive parenchyma,** interspersed among the sieve-tubes.

As the vascular bundles of the rhizome of *Pteris* (the common Bracken) are more regularly typical of the concentric bundle of the Fern, than those of *Aspidium*, thin transverse sections should also be prepared from the rhizome of this plant, or the rhizome of *Pteris* may be taken as a substitute for the stock of *Aspidium;* mount some of the sections in glycerine, others in chlor-zinc-iodine; or these sections may be stained with hæmatoxylin and mounted in Canada balsam (p. 52).

First examine the whole section under a low power, and note its oval form, the upper and lower surfaces being flattened. It is composed of the following tissues:—

1. A peripheral band of brown **sclerenchyma,** outside which is a scarcely distinguishable epidermis.

2. Colourless, parenchymatous **ground-tissue,** in which are embedded successively the following:—

3. An outer series of relatively small, sharply circumscribed **vascular bundles,** arranged in an irregular ring: one larger bundle is usually to be found opposite the lower flattened surface.

4. An incomplete ring of dark brown **sclerenchyma,** composed of two parts which are usually distinct, viz. an upper strongly curved portion, and smaller flat portion which is nearer the lower surface: small isolated patches of brown sclerenchyma may also be seen dotted about in the ground-tissue.

5. A **central series of bundles,** usually two or three in number, and of larger size: they may sometimes be found to be connected at their margin into one irregular ring-like mass.

Examine the **sclerenchyma** under a high power, and note the

thick, brown, stratified walls: the cells contain a somewhat reduced protoplasmic body.

Examine also the parenchymatous **ground-tissue,** which will be found to consist of cells with thin cellulose walls, and mucilaginous protoplasmic contents, with much starch. Note especially small pegs and rods, which appear as superficial outgrowths from the walls which adjoin the intercellular spaces.

Select a single elliptical vascular bundle for detailed observation under a high power, and recognize—

1. The **bundle-sheath** or **endodermis,** which is a definite, but narrow layer of cells, with brown coloured contents.

2. The **phloem-sheath** or **pericycle,** which is not quite so definite a layer of deeper cells, with cellulose walls, and plentiful protoplasm.

3. Within this is a band of **phloem,** which is wider at the flattened sides of the bundle, but narrower at the two poles: it consists of—

a. **Sieve-tubes,** which may be recognized by their large cavity, sparing contents, and cellulose walls: note where two sieve-tubes are contiguous that bright yellow granules may be seen adhering to the wall: these indicate the position of the **sieve-plates.**

b. Parenchymatous cells, which fall under the general term of **conjunctive parenchyma.**

4. An elliptical area of **xylem** occupies the centre of the bundle, it is composed of—

a. **Tracheides,** which are large and of circular or polygonal outline, with highly refractive lignified walls, and no cell-contents. Towards the poles of the elliptical area may be seen tracheides of small size, and circular outline: these are the **protoxylem.**

b. **Conjunctive parenchyma,** distributed irregularly among the tracheides.

Longitudinal sections should also be cut, so as to traverse the vascular bundles of the central series: they may be treated as above directed. Note in these the thin-walled **ground-parenchyma,** and the brown **sclerenchyma;** the latter consists of fibrous cells with peculiar **crossed pits** in their walls.

a. The **bundle-sheath,** or **endodermis,** consists of narrow oblong cells with square ends, and with brown contents.

b. The **phloem-sheath,** or **pericycle,** is composed of wider oblong cells, with colourless protoplasic contents : the ends are square or oblique.

c. The **sieve-tubes** appear as wide tubes, with pointed ends, and cellulose walls : the surfaces of the walls which separate contiguous sieve-tubes are covered with numerous **sieve-plates** (best seen in sections treated with chlor-zinc-iodine), to which round, highly refractive granules adhere : the sieve-tubes are in fact essentially similar to those of *Aspidium* (p. 297). Note especially the irregular outline of the wall when seen in longitudinal section : this will be best seen in sections which have been cut so as to pass through the phloem in a plane parallel to the flattened side of one of the larger bundles.

d. The **scalariform tracheides,** which are the main constituents of the xylem : they are similar in all their main characters to those of *Aspidium* (see p. 297), being elongated and pointed, while the walls are marked by transversely-extended bordered pits, arranged regularly so as to give the ladder-like appearance ; but they differ from them in one point, for by a careful examination of fine sections it may be ascertained that the pit-membrane, which remains permanently in *Aspidium*, is often broken down on the oblique terminal walls in *Pteris* : this is, however, exceptional for Ferns.

e. The **conjunctive parenchyma,** distributed among both sieve-tubes and tracheides.

X. From around the apical bud of a well-grown plant of the Male Fern remove successively the bases of the leaves of previous years, those of the current year, and finally the larger circinate leaves, which would have unfolded in the following year. Carefully remove the smaller ones with a scalpel, and then with forceps gradually pull off the large mass of brown scales, which completely cover the extreme apex. With a stiff camel's-hair brush remove the bases of these scales, together with the youngest of them, which will still remain

round the *punctum vegetationis:* after this treatment it will be easy to observe with a pocket lens—

1. The **apical cone** (*punctum vegetationis*), a rounded papilla, occupying a central and terminal position in the flattened apical region.

2. The **young leaves,** situated round the apical cone, and successively larger the further they are from the apex. Note the circinate curvature which appears at an early period in their development.

XI. With a sharp razor, wet with water or with very weak spirit if the material be fresh, or with strong

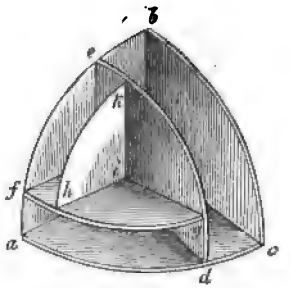

FIG. 13.—View of a model of a three-sided pyramidal (or tetrahedral) apical cell, as seen from above: the walls *d e*, *f g*, *h k*, denote successive walls by which segments have been cut off from the growing apical cell: *i* is the apex of the pyramidal cell, at which point the three youngest segmental walls cut one another. (After Sachs.)

spirit if it has been previously hardened in alcohol, remove the extreme apex of the *punctum vegetationis*, taking care to cut accurately in a transverse plane: mount in water, or in weak glycerine, and examine with a low power. If the section be thin enough, it will be seen that a large cell of **triangular outline** occupies the centre of the apical cone, while the cells

immediately surrounding it are arranged in more regular order than those at a greater distance. This cell is the **apical cell,** and the cells surrounding it have been derived by cell-division from it, by means of walls parallel to its three sides: they are called the **segmental cells,** and it may readily be seen that these again undergo subdivision. If the section be not sufficiently transparent, it may be treated with very dilute potash and weak glycerine, or, better, with "eau de javelle," which will clarify the tissues, and make the cell-walls more distinct.

The form of the apical cell, and of the segmental cells which surround it, will be readily appreciated on comparison of Fig. 13.

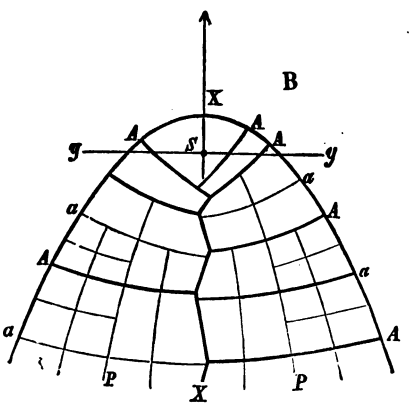

FIG. 14.—Diagram showing the arrangement of cell-walls as seen in a median longitudinal section through an apical cone with a pyramidal apical cell. *A A*, are the segmental walls, which form part of the system of anticlinals; *a*, *a*, walls by which each segment is cut into two equal halves: these complete the anticlinal curves; *P P*, periclinals, which are not completed up to the apex. (After Sachs.)

XII. From the apex of another plant cut median longitudinal sections: mount in weak glycerine: a

little very dilute potash may be added if the sections are not transparent enough, or they may be treated first with "eau de javelle," and then be mounted in glycerine.

If any one of the sections has passed through the apical cone, in a median plane, the **apical cell** will be seen presenting a wedge-like appearance, and the cells around it will show, in the regularity of their arrangement, that they have been derived from segments successively cut off from the apical cell. (Compare Fig. 14.) It may be concluded from the observation of transverse and median longitudinal sections that the form of the apical cell is that of a three-sided pyramid.

The structure and mode of origin of the young leaves should also be observed in the median longitudinal sections.

Similar preparations should also be made from the apex of the rhizome of *Pteris:* in these a single apical cell may be found, having the form of a two-sided cone (*i.e.* like half of a biconvex lens).

The Root.

XIII. Cut transverse sections of the root of the Male Fern, selecting for that purpose the thickest part of an old root: mount in glycerine, and observe—

1. The **piliferous layer:** a single layer of superficial cells have grown out as root-hairs, remnants of which may still be seen.

2. The greater part of the section consists of the bulky, brown-walled **cortex,** of which the outer parts

are thin-walled ; but, passing inwards, there is a sudden increase in thickness of the wall, so as to form a dense **sclerenchymatous ring** : this surrounds—

3. The **endodermis,** which consists of a single layer of cells flattened tangentially, and having the usual dotted marking of the radial walls : this may be difficult to observe, as the radial walls are often pressed out of shape. Within this layer lies—

4. The **pericycle,** which usually consists of two layers of cells, with thin walls, and obvious protoplasmic contents. The vascular tissues inclosed within these layers are arranged according to the ordinary radial type ; thus there will be seen—

5. Two groups of **xylem** abutting on the pericycle and composed of **tracheides** of various size, the largest being near the centre of the root : as the root develops, the two originally separate groups of xylem unite at the centre by formation of fresh tracheides, and together form a flat band which traverses the root longitudinally. Alternating with the groups of primary xylem at the periphery of the vascular cylinder are—

6. Two groups of **phloem,** consisting mainly of **sieve-tubes** having the same characters as those of the stem. Scattered among the vascular elements are cells of **conjunctive parenchyma.**

Note that one or two cells of the endodermis opposite the groups of xylem are larger than the rest : these are the **rhizogenic cells,** which might have been the starting-points of lateral roots : the latter are formed at an early stage of development of the tissues of the root, *i.e.* near to the apex ; if transverse sections be made through the young part of a root, lateral roots may be

found in course of development in positions corresponding to the rhizogenic cells.

If transverse sections of the root be cut at a point not far removed from the apex, it will be seen that the xylem is not yet developed at the central part of the vascular cylinder while the peripheral parts may be fully formed: thus the development of the xylem is centripetal, the root is diarch, and the arrangement of the vascular tissues is radial.

XIV. Cut median longitudinal sections of the apex of a root which has been hardened in alcohol: at most only one absolutely median section can be obtained from a single root: it will be found convenient to embed the apex of the root in paraffin, or to hold it between pieces of pith or carrot. Mount in glycerine, and examine first with a low power: choose out those sections in which there is a symmetrical arrangement of tissues around a single, large, apparently three-cornered **apical cell,** which lies at some distance from the extreme apex. (Compare Fig. 14.) Note—

1. That the orientation of the apical cell is constant, *i.e.* one corner is directed towards the older part of the root, while the side opposite that corner, *i.e.* the anterior face or **base** of the cell, is perpendicular to the axis of the root.

2. That around the apical cell are regularly arranged **segmental cells,** which have successively been cut off from it by walls parallel to the sides of the apical cell. Of these—

a. Those successively cut off from the base form the **root-cap,** dividing up by regularly arranged walls into a mass of regular cells.

b. Those cut off from the sides of the apical cell form the body of the root: these also divide by walls in regular succession. Observe carefully the arrangement of these walls, and by comparison of several sections ascertain their order of succession, and their relation to the various tissues of the root above described.

XV. Cut successive transverse sections of the apex of a root which has been hardened in alcohol: this

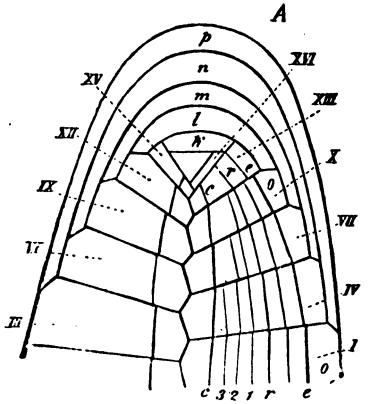

FIG. 15.—Diagram illustrating the arrangement of cell-walls as seen in a median longitudinal section through the apex of the root of a Fern. *I. III.* &c., indicate segments cut off from the sides of the apical cell, which go to form the body of the root; *k*, *l*, *m*, *n*, *p*, are successive segments from its base, which go to form the root-cap. (After Goebel.)

may easily be done if the root be held between pieces of pith, or better by embedding in paraffin. If possible, keep all the sections in their proper order of succession, and mount in glycerine. Examine with a low power, and choose out those in which the large apical cell is to be seen. Observe carefully—

1. The **form** of the **apical cell,** apparently **three-sided:** combining this result with that obtained by examination of the longitudinal sections the form of the whole cell must be a **three-sided pyramid.** (Compare Fig. 13.)

2. The **segments** are arranged in regular order round it, and are cut off successively from the three sides.

3. Note the mode in which the several segments are further divided.

Next examine a section which has passed through the root-cap **immediately above the apical cell:** this will include the young segments cut off from the base of the apical cell by transverse walls, and destined to form the root-cap. Note the first divisions of these segments by walls arranged crosswise: it may be seen that these walls do not coincide in position in successive segments.

The Leaf.

XVI. Cut transverse sections of a pinna of a leaf of the Male Fern which has no sori upon it: mount in weak glycerine, and observe with a low power that the outline of the section shows the leaf to be of equal thickness throughout, except where traversed by vascular bundles: at those points the pinna is thickened, the lower surface projecting convexly.

Examine with a high power, and observe successively the following tissues, starting from the upper surface:—

1. A regular **epidermis** with a thin cuticle: the

epidermal cells contain chlorophyll: there are no stomata.

2. The **mesophyll** consists in its upper part of thin-walled cells containing chlorophyll, and with small intercellular spaces; this passes by gradual transition into the lower part, where the intercellular spaces are larger, and the form of the cells less regular. Internal glandular hairs are frequently to be found in the intercellular spaces of the mesophyll.

3. The lower **epidermis,** the cells of which also contain chlorophyll: numerous **stomata** are present: note the form of the two **guard-cells** as seen in transverse section, and their position in relation to the epidermis.

4. Here and there **vascular bundles,** of circular appearance in transverse section, will be found embedded in the mesophyll: the larger of these correspond in position to the swollen ribs of the pinna.

Note the **endodermis** as a continuous layer of cells, which completely surrounds the circular bundle, and within this the xylem and phloem elements, similar to those of the stem: the bundles show a tendency to the **collateral** type, the xylem being nearest to the upper surface of the leaf.

XVII. Cut tangential sections (or strip off the epidermis) from the upper and the lower surface of the leaf: mount as before, and compare them.

a. The epidermis of the **upper surface** will be found to consist of cells with sinuous outline, and protoplasmic contents, with chlorophyll: **no stomata** will be found.

b. The epidermis of the **lower surface** consists of

cells similar to the above: there are **stomata** with two guard-cells.

The development of these stomata may be studied with advantage in young leaves, by stripping off the epidermis, or by cutting tangential sections. Special attention may be given to the peculiar case of *Aneimia*, in which the mother-cell of a stoma is cut off from an epidermal cell by a cylindrical wall, as a circular hole is cut in a sheet of wadding by a wad-punch.

The Sporangia.

XVIII. Cut transverse sections through pinnæ of leaves which bear **sori**, taking care that the sections shall pass through one or more sori: mount as before, and examine with a low power. Note—

1. The structure of the pinna, as above described.

2. Opposite to, and seated upon a rib will be found the membranous **indusium**, which, like an umbrella, covers over—

3. The **sporangia**, which are biconvex-lens-shaped, brown, stalked capsules, attached to the rib, and filled with—

4. Numerous roundish, brown, unicellular **spores**.

Observe more closely the structure of the single sporangium. It is composed of—

i. The **stalk**, which is of considerable length, and usually consists of three rows of cells.

Stalked glandular bodies are often found as lateral branches on the stalk of the sporangium in this species.

ii. The **capsule**, which has the form of a biconvex lens, and consists of a marginal series of cells with

peculiarly thickened walls, which constitute the **ring,** or annulus, and thinner-walled, flattened cells, which together form the lateral walls of the completely closed sporangium.

Place a number of mature, but not yet ruptured sporangia upon a dry slide : warm them very gently over a spirit-lamp, and observe quickly under a low power : note the sudden explosive rupture of the sporangia, so as to eject and scatter the spores : this is due to the straightening of the annulus or ring. Similar results may be obtained by mounting in water, and subsequently adding glycerine ; in fact, on the removal of water by evaporation into the air, or by a reagent such as glycerine, the curved annulus tends to straighten itself, and then ruptures the thin wall of the sporangium.

Note sporangia in which the thin lateral walls have been ruptured transversely, the ring having straightened itself out : now breathe on the sporangia, and note that on being thus moistened by the breath the annulus becomes more curved, while on being left exposed to the dry air for a few minutes it again becomes straight.

Examine single spores under a high power : they are **unicellular** bodies, having a brown wall, with external band-like outgrowths of the exospore or outer layer of the wall. All the spores are alike (Homosporous).

The various stages of development of the sporangium may be found in any sorus in which only the first sporangia have come to maturity : treat the sections previously with weak potash ; if this makes them too transparent, neutralize with weak acetic acid, and mount in glycerine ; or the sections may be treated at once with " eau de javelle, " and then be mounted in glycerine.

Note especially in such sori the following stages of development:—

i. The sporangium appears as a simple, hair-like process, consisting of a single cell, or of two separated by a transverse wall.

ii. The upper cell divides so as to consist of a central tetrahedral cell (**archesporium**), surrounded by a single layer of cells, which form the wall.

iii. The central cell or archesporium is divided into—

a. One cell, or ultimately a group of cells, lying centrally, which give rise to the **mother-cells of the spores**, and finally, by division of each mother-cell into four, to the spores themselves.

b. A layer of transitory **tapetal cells**, which surround *a*, and are ultimately absorbed.

B.—THE GAMETOPHYTE, OR OOPHYTE.

I. Dry some of the leaves of the Male Fern, which bear sori, on a piece of paper: the spores will then be set free by the rupture of the sporangia, and they may thus be collected in large quantities. Sow some of them on damp earth: keep them moist, and sheltered from direct sunlight; they will then germinate, and after a few weeks the surface of the soil will be found to be covered with small, green, flattened bodies, each of which is an individual **prothallus.**

If it be desired to follow the germination of the spore, and the first stages of the development of the prothallus in detail, the spores may be placed in a hanging drop in a moist chamber, as described in Appendix A. But for all ordinary purposes it will suffice to pick off young prothalli, from time to time, with a needle, from the surface of the soil on which spores have previously been sown: by this means a series of preparations illustrating the various stages of development of the prothallus may

be obtained. Note in such a series of preparations, under a low power—

1. The bursting of the outer coat of the spore, and the protrusion of the inner coat through the slit.

2. The formation of—

a. An aërial portion, containing chlorophyll, and undergoing repeated cell-divisions, which result in the development of a flattened, roughly triangular, expansion.

b. A root-hair, which remains undivided, does not contain chlorophyll, and grows downwards into the soil.

3. In older prothalli of the series note an incurving of the margin of the part more remote from the original spore: this is due to the slower growth of that part and the more rapid growth of the lateral parts: at the base of this depression is one wedge-shaped **apical cell,** from which segments are cut off alternately on opposite sides. The identity of the apical cell and regularity of the segments are lost in the later stages of development.

II. Examine a single, fully-grown prothallus with the naked eye, and observe—

1. The **form,** which is flattened, and more or less kidney-shaped, with a depression of the margin, at the base of which is the **organic apex** of the prothallus. Note that the central part of the prothallus is often perceptibly thicker than the periphery: this thicker part is called the **cushion.**

2. The **position** of the prothallus while growing: it is usually oblique to the surface of the soil.

3. The **root-hairs,** which spring from the under surface of the cushion, and run downwards into the soil.

4. The **green-colour,** due to the presence of chlorophyll: the prothallus is thus capable, under suitable circumstances, of carrying on the process of elaboration of fresh organic substances.

III. Wash a fresh, well-developed prothallus carefully in water, so as to remove the soil from the root-hairs: mount it whole in water, with the lower surface directed upwards, and examine it with a low power. Observe again the chief points seen above with the naked eye, which are now more plainly visible, and note especially—

1. The **form** and **structure** of the cells in the lateral, thinner portions of the prothallus; they are polygonal, and have thin cellulose walls, and protoplasm containing a nucleus and numerous chlorophyll-corpuscles: the cells at the margin are often extended as hair-like outgrowths.

2. The cells composing the cushion are of similar structure, but are aggregated in a mass more than one layer of cells in thickness: many of the cells will be seen to have grown out as root-hairs.

3. The depressed **apex** of the prothallus, which is occupied, not by a single wedge-shaped cell, as is the case in early stages of development, but by a closely aggregated series of marginal cells, with thin cell-walls, and every appearance of recent and repeated cell-divisions.

4. The **antheridia,** which are hemispherical outgrowths, situated chiefly on the posterior and lateral portions of the underside of the prothallus.

5. The **archegonia,** which are situated on the cushion near to the organic apex of the prothallus; the multicellular **neck** of the archegonium projects from the surface of the prothallus as an elongated cylindrical structure.

Under the low power select one mature antheridium,

and, without moving the slide, adjust the higher power so as to observe the structure of the same antheridium in detail. It will then be seen that it consists of—

a. A **wall,** composed of a single layer of narrow cells ; this completely surrounds—

b. The **spermatocytes,** or mother-cells of the spermatozoids, which are small, and not very numerous.

Other antheridia may be found which have already burst the outer wall: in these the contents of the mother-cells may perhaps be seen escaping from the ruptured antheridium as spiral **spermatozoids,** endowed with active movements.

If a preparation showing motile spermatozoids be treated with a weak solution of iodine, the movements will cease with the death of the spermatozoids, which will assume a brown staining, while the **cilia** attached to the anterior ends of them will then be clearly seen.

Select under the low power one mature **archegonium,** and then observe it in detail under the higher power. If the neck be vertical, which would under the circumstances be the natural position, since the prothallus was mounted with the lower surface uppermost, there will then be seen, on focussing down upon it, **four** rows of cells composing the wall of the neck, and surrounding one cell, the **canal-cell.**

IV. Treat some prothalli with a saturated solution of picric acid in water for some hours : wash them with water, and then harden them gradually by successive treatment with alcohol of 50 per cent., 70 per cent., and finally with absolute alcohol or strong methylated spirit; or the material may be hardened at once in

absolute alcohol, a method which gives very good results. The preparations described below may also be made from fresh material, but the results will not be nearly so good as if one of the above methods of fixing and hardening be adopted.

Hold a prothallus thus prepared between pieces of pith, or embed as directed on p. 11 : then cut sections perpendicularly to the surface of the prothallus, and so as to pass through the cushion, following the organic axis from base to apex. Mount in glycerine, and examine first with a low power.

The **lower surface** may easily be recognized by the presence of **root-hairs** : on this lower side, chiefly near to the apical end of the section, which is characterized by its small cells with thin walls, will be found **archegonia** : these may be recognized by the multicellular **neck,** which projects beyond the surface of the section. In some cases the canal of the neck may appear of a deep brown colour : this is the case in old archegonia which have not been fertilized, and they must be disregarded. Select one **archegonium** of full size and healthy appearance, and examine it under a high power.

Observe that it consists of—

A. The **central series** of three cells, which may be distinguished as—

a. The **canal-cell** : this is oblong in form, and its walls are subject to mucilaginous degeneration : it occupies the channel of the neck, and has been above alluded to as being visible when the neck of the archegonium is seen from above.

b. The small **ventral canal-cell,** which lies imme-

diately below the oblong canal-cell, and is of rounded form.

c. The **oosphere,** or **ovum,** which is of relatively large size, and roughly spherical form: it is embedded in the tissue of the cushion, and consists of a dense mass of granular protoplasm.

B. The **neck,** which is composed of cells arranged in four rows, constituting together a cylinder or tube, one layer of cells in thickness: this projects from the surface of the prothallus, and incloses the cells (*a*) and (*b*) of the central series, while (*c*), the ovum, is embedded in, and surrounded by, cells of the cushion.

At the end of the section more remote from the apex may be found **antheridia.** Select one fully developed, and it will be seen to consist essentially of an outer wall, one layer of cells in thickness, which incloses a central mass of cells, the contents of which may be seen to be rounded off, and to have assumed the form of a closely coiled spiral: these are the **spermatocytes** or mother-cells of the spermatozoids.

By comparing carefully-prepared and well-cut sections, the development of the antheridia and archegonia may be traced, and in both cases it may be seen that they originate from single superficial cells. In the case of the antheridia young stages of development are to be found on sections through the lateral and posterior parts of the prothallus, while young stages of development of the archegonia lie near to the organic apex: these preparations may be cleared with "eau de javelle." Young archegonia should also be observed from above in young prothalli mounted with the lower surface uppermost. If drawings be made of archegonia from both points of view, and of various ages, a comparison of them will give a clear idea of the processes of development.

The dehiscence of the antheridia, the escape of the spermatozoids, and their movement, should be observed with particular attention in fresh prothalli mounted in water; also the opening of the apex of the neck of the archegonia; in both cases the process depends upon a mucilaginous degeneration of cell-walls of the inner cells, and a subsequent swelling by taking up water, and consequent rupture of the outer walls. Further, the movements of the living spermatozoids may be followed, and the act of fertilization observed: the spermatozoids pass through the mass of mucilage which fills the neck of the archegonium, and finally coalesce with the ovum.

C.—THE YOUNG SPOROPHYTE, OR FERN-PLANT.

I. The result of the process of fertilization of the ovum of the archegonium by the spermatozoids is the development of a new Fern-plant (the sporophyte), and in cultures which have been continued for some months such young Fern-plants may be clearly seen attached to the prothalli, but one prothallus produces only one young Fern-plant.

Select a prothallus to which a young Fern-plant is thus attached, and wash from it the soil which adheres to it. Examine it with a lens, and observe—

1. That the prothallus itself is similar in form and structure to those before observed.

2. That the young Fern-plant is firmly attached to its under surface by a lateral protrusion (**foot**).

3. That the young Fern-plant consists of the following parts:—

a. A **root,** which turns downward into the soil.

b. A lateral protrusion, the **foot,** which maintains

a close physiological connection between the prothallus and the Fern-plant.

c. A first leaf, or **cotyledon,** with an elongated petiole, and bifurcating, expanded, upper part: this usually grows upwards through the depression at the apex of the prothallus.

d. Between the base of the cotyledon and the foot is the **apex of the stem,** which continues its growth, and produces new leaves.

Having thus gained a knowledge of the position of the several parts relatively to one another, and to the prothallus, in the case of a young Fern-plant of considerable size, younger plants may successively be taken, and by a comparison of these the mode of **development** of the young embryo, or Fern-plant, may be traced. In order to make preparations so as to show the several parts of the embryo, sections should be made either from fresh material, or better from material prepared with picric acid, and hardened in alcohol, as above directed for the prothalli. The direction of section should be parallel to the organic axis of the prothallus, and perpendicular to the flattened surface: in such sections, including embryos of suitable age, the stem, cotyledon, root, and foot may all be observed; further, the origin of these several parts from definite segments of the fertilized ovum may be traced. For details as to the sequence of cell-divisions in the first stages of development of the embryo, reference should be made to text-books.

II. Heterosporous Type

PILULARIA GLOBULIFERA (The Pill-Wort)

1. *External Characters*

I. This plant is to be found, but not very commonly, growing on the edges of moorland ponds: owing to its small size, and to the appearance of its leaves, it may often be passed over, as it frequently grows among other plants of aquatic habit from which it is not readily distinguished at first sight. Examine specimens of the plant with the naked eye, and note—

1. The cylindrical, green, creeping **rhizome,** with an upturned apex, which bears—

2. Simple cylindrical vertically growing **leaves:** note their alternate arrangement, and lateral insertion on the axis; also their crozier-like or circinate vernation.

3. One or more brown **roots** are attached to the under side of the rhizome at points below each of the successive leaves.

4. **Lateral branches,** which repeat the characters of the main axis, are attached beside and slightly below the leaves, but their position is not axillary.

5. In the autumn, round, brown, pill-like bodies, the **sporocarps,** are to be found attached by short stalks to the axis, and apparently in an axillary position.

II. *Microscopic Investigation*

II. Cut transverse sections of the rhizome of *Pilularia*, mount in glycerine, others in chlor-zinc-iodine, and observe—

1. The **epidermis,** with a stratified outer wall, and definite cuticle : stomata are to be found.

2. The parenchymatous and starchy **cortex,** containing large intercellular air-spaces, separated by radiating diaphragms one layer of cells in thickness.

3. The brown, well-defined **endodermis,** which surrounds—

4. The cylindrical **vascular bundle.**

5. Centrally may be seen a mass of **sclerenchyma** surrounded and marked off from the bundle itself by an inner **endodermis.**

This is the type of structure for strongly grown rhizomes, but where the rhizome is weak the inner endodermis and sclerenchyma may be absent, and the vascular tissue then appears as a solid strand.

Examine the constituents of the bundle more carefully, and note a circular series of **tracheides** similar to those in the xylem of other Ferns: this is surrounded on either side by phloem, which is also similar to that of other Ferns.

Longitudinal sections of the same should also be made, and the structure of the elements of the bundle compared with that in homosporous Ferns. It is to be noted that *Pilularia* is a plant of aquatic habit, and shows, in the presence of large intercellular cavities, and in the reduced character of the vascular tissues,

a similarity to aquatic Phanerogams (compare p. 110, 171) and Equiseta.

Cut also transverse sections of the rhizome of *Marsilea:* it will be found to show a similar general plan of structure, but it is more robust than *Pilularia:* sclerenchyma is found in the cortex, and the xylem and phloem are more largely developed, and better differentiated: the cylindrical form of the vascular bundle corresponds to that of *Pilularia*, but the ring of vascular tissue is here usually wider, and it will serve as a link to connect these reduced bundle-systems with those of such as *Aspidium* which have an open meshwork.

III. Cut transverse sections of the leaf of *Pilularia:* and, treating as before, note the circular form of the section: this is a most perfect centric type of leaf. Examine and observe—

1. The **epidermis,** with numerous stomata.

2. The **mesophyll,** consisting of a single hypodermal layer, within which are large intercellular cavities separated by radiating trabeculæ, one layer of cells in thickness, and partitioned by transverse diaphragms.

3. A central, cylindrical, **vascular bundle** with a definite endodermis: compare the structure of the bundle with that of one of the bundles of the stem.

IV. Cut transverse sections of the root, and observe in them—

1. The **epidermis,** which in old roots is squeezed out of shape.

2. The **cortex,** which is composed of (*a*) an outer hypodermal layer; (*b*) radiating lamellæ which separate large intercellular spaces: single cells of these lamellæ occasionally grow into the cavities as large spirally coiled **internal hairs,** which probably serve to

strengthen the superficial tissues; (*c*) two internal layers, the inner of which has thickened brown walls.

3. The **endodermis,** consisting of narrow cells.

4. The central **vascular cylinder,** similar in general arrangement to that of other Ferns, but of a reduced type: usually only eight elements of xylem, very regularly arranged to form a diarch plate, are to be seen in the transverse section: the phloem is very much reduced.

Preparations should also be made of the apex of the stem, and of the root as above directed for other Ferns.

V. Cut transverse sections of the mature sporocarp, these are to be found in large numbers on the plant in August and September: mount in pure glycerine, and examine under a low power.

The circular section will be found limited by a thick and firm **wall,** bearing external hairs: the internal space is partitioned into four **loculi** by septa of thin-walled tissue. Projecting from the outer wall into each loculus is a cushion-like placenta, upon which are inserted the numerous **sporangia**: these are of two sorts, **macrosporangia,** which contain each a single large **macrospore,** and **microsporangia,** each containing numerous smaller **microspores.**

Examine the section under a high power, and observe that the wall of the sporocarp consists of—

a. A very irregular layer of **epidermis,** with **stomata,** having two depressed guard-cells: note also the insertion of the superficial hairs.

b. Two layers of very regular **prismatic cells,** closely

packed, and with remarkably thickened and stratified walls.

c. Within these is a **spongy parenchyma,** with large intercellular spaces, and much starch: concentric vascular bundles are found here and there embedded in it.

d. The **placentæ,** consisting of parenchyma, with a large vascular bundle in the centre of each.

e. The **microsporangia,** each consisting of a short stalk, and an elongated pear-shaped head: the wall, consisting of a single layer of cells, may be more or less disorganized according to age, the cell-walls being mucilaginous: the **microspores** are numerous, and relatively small: their wall consists of an inner firm layer, and an outer stratified, mucilaginous layer, which swells largely with water. Centrally is an undivided protoplasmic body.

f. The **macrosporangia** have an outer wall consisting of a single layer of cells, which closely invests the single large **macrospore:** this has its apex directed away from the placenta. Centrally is a large, undivided protoplasmic body, with numerous large starch-grains: this is surrounded by a massive wall, in which four layers may be recognized: the inmost layer is thin and brown: the next layer is also thin, and projects as the apical papilla: the third layer forms an incomplete covering of variable thickness, and very marked prismatic structure: the fourth, also incomplete at the apex, is a very wide mucilaginous band, showing indications of striation and stratification.

By cutting sporocarps of various ages taken earlier in the year, the development of the sporangium may be found to coincide in

its early stages with that of homosporous leptosporangiate Ferns: it is only in the later stages, after the division of the spore-mother-cells, that the distinction appears between male and female sporangia.

By keeping mature sporocarps moist during the autumn, their rupture by four valves may be observed, and the extrusion of the macro- and microspores embedded in a swollen mass of mucilage: in this the spores germinate, but it disappears after a time. Cultures should be kept, and examined at intervals.

Longitudinal sections should be cut through the macrospores after the mucilaginous mass has disappeared: in these the rudimentary female prothallium may be found at the apex of the spore, bearing an archegonium: in others, taken later, young embryos in various stages of development may be found and examined. Compare these with those of other Ferns.

Observations may also be made with advantage on the sporocarp of *Marsilea*, which, though differing in form from that of *Pilularia*, corresponds to it in its essential points.

C.—EQUISETINEÆ

EQUISETUM ARVENSE (The Common Horse-Tail)

THE SPOROPHYTE

External Characters

I. Observe with the naked eye the following external characters in specimens of *E. arvense*, which is the common Horse-tail of corn-fields and road-sides: its green vegetative shoots are to be found from early summer onwards. The root-stock being a creeping one, and underground, it cannot be removed from the soil without injury by merely pulling it up: the specimens should be carefully dug up, so that the several parts may be seen in their natural position relatively to one another.

1. The external conformation of the **axial structures** or stems is the same whether they be creeping and underground, or erect and aërial: they consist of more or less elongated joints or **internodes**, marked off from one another by **nodes**, which may be recognized as the points of insertion of—

2. The **leaf-sheaths**, each of which surrounds the base of the internode next above it, and splits at its

upper limit into **teeth,** the number of which varies on different axes.

3. The internodes are marked by projecting longitudinal **ridges,** which may be traced upwards into the leaf-sheath, and are then seen to be continuous to the apices of the teeth: between the ridges are depressed channels.

4. The **lateral branches** are always inserted at the nodes, and at the base of the leaf-sheaths: note that they are arranged in whorls, and appear to burst through and rupture the leaf-sheath near to its point of insertion on the axis, and at points alternating with the projecting ridges, *i.e.* at the channels.

5. The **roots** (to be clearly distinguished from the underground root-stock, which shows an alternation of nodes and internodes as above described) are thin and fibrous, and branch **monopodially:** they are inserted with a whorled arrangement at the nodes, immediately below the point of insertion of the lateral buds. The underground stems and the roots are covered externally by numerous fine root-hairs of a brown colour.

6. Note that at many of the nodes the lateral branches, or the roots, or both, may be partially suppressed, their development being arrested at an early stage: also that frequently the basal internode of lateral shoots attached to the nodes of the root-stock may be much distended, while its apical bud is arrested: in some cases more than one internode may take part in this development, the result being a moniliform structure: the **tubers** thus formed are reservoirs of reserve material, and being easily separated from the parent plant, they

serve to propagate the plant by a purely vegetative process. Any node separated from the parent plant may also serve the same purpose under favourable circumstances.

7. Observe particularly that the teeth of each leaf-sheath correspond in position to the channels of the next higher internode: since the teeth are continuous downwards with the ridges of the lower internode, it follows that the ridges of the lower internode alternate in position with those of the internode next above it.

Strip off carefully one leaf-sheath, and it may then be clearly seen that the ridges of the upper internode alternate with those of the internode next below it.

Microscopic Investigation

II. Cut transverse sections from a mature internode of an upright aërial stem: mount some in glycerine, others in chlor-zinc-iodine, and examine first with a low power: observe—

i. The sinuous outline of the section, the projections corresponding to the ridges observed externally with the naked eye, and the indentations to the channels intervening between them.

ii. The section is limited at the periphery by an ill-defined layer of epidermis, which, together with subjacent tissues, forms a band of thick-walled tissue of very variable breadth; thus the band is broad at the most convex part of the ridges, and at the most de-

pressed parts of the channels, while it is reduced on the sloping sides of the ridges to the single layer of thick-walled epidermal cells.

iii. Beneath this is a broad band of **cortical tissue,** in which may be recognized—

a. Groups of **chlorophyll-parenchyma** of oval outline: one of these lies on each slope of the furrows, and extends to points close below the surface of the sloping sides.

b. Parenchyma composed of rounded cells with little or no chlorophyll.

c. Large **intercellular cavities,** which alternate in position with the ridges, and are thus opposite the channels of the outer surface.

iv. The cortex is limited internally by a single sinuous layer consisting of cells in close contact with one another: this is the **endodermis:** it forms a continuous and sinuous ring surrounding—

v. The **vascular bundles,** which may be recognized as oval groups of elements of smaller size than those of the surrounding tissue: they alternate in position with the intercellular cavities of the cortex, and are thus opposite to the ridges which project on the external surface.

vi. The **pith,** which lies centrally, consists of thin-walled tissue, and is in great part obliterated by a large central cavity.

III. Before proceeding to the more minute study of these several tissues, cut transverse sections through a leaf-sheath: mount in glycerine, and examine with a low power. It may be observed that the arrangement of tissues is not unlike that of the peripheral tissues

of the internode. Note especially that, as in the internode, so also in the leaf-sheath, one vascular bundle, here of small size and simple structure, is to be found opposite each ridge.

IV. Cut a series of rather thick transverse sections through the nodal region: it will be best to select one which bears no fully-developed, lateral branches. Keep them all in their right order of succession, mount, and compare them under a lower power, starting from such a section above the node as will show an arrangement of tissues typical of the internode, together with the leaf-sheath surrounding it. If these parts be in their natural position, it will be seen in this first section that the vascular bundles of the leaf-sheath alternate in position with those of the internodes. Passing the sections successively under observation, it will be seen that each bundle of the internode divides into two branches, which diverge, and insert themselves respectively right and left on bundles of the leaf-sheath, at the point where these curve into the axis, and begin their downward course through the next internode. Thus the course of each bundle of the leaf-trace is simply this: it passes from the leaf-sheath into the axis, traverses one internode, and at the next lower node it forks, the two equal branch-bundles inserting themselves, right and left, on bundles entering at that node.

These facts, together with external observation of the ridges of the leaf-sheaths and internodes, will suffice for the construction of the bundle-system of the shoot of *E. arvense*, which is also typical of the whole group.

The bundle-system may be actually demonstrated by dissection in the stem of one of the larger forms, viz. *E. Telmateia.* Take a fresh and well-grown shoot, and cut from the thickest part of it a piece about four inches in length, and including a node ; then remove from it the outer tissues, and scrape the soft parenchyma away till the vascular bundles are laid bare ; then slit the hollow stem longitudinally, flatten it out, and carefully scrape away the softer tissues from the inside till the vascular bundles are clearly seen ; then treat for some hours with alcohol to remove the air-bubbles from the intercellular spaces, and warm gently in weak solution of potash : the preparation may be preserved in glycerine, or alcohol. If such a preparation be carefully made, it will show the course of the vascular bundles in the internode, as well as the branchings and fusions at the node.

Returning to the study of the details of the transverse sections of the internode, examine them under a high power, and observe—

1. The superficial cells of irregular size and shape which form an ill-defined epidermis, many projecting as rounded excrescences beyond the general surface : their walls are thick, and show on the outer surface small and irregular projections : the cell-contents are scanty. Note that on the sloping sides of the ridges, and immediately above the chlorophyll-parenchyma, **stomata** may be seen cut in section, and showing two **guard-cells** which surround the pore, and two **subsidiary cells** which fit closely round them : there is a large respiratory cavity beneath each stoma.

2. The subjacent cells, composing with the epidermis the band of thick-walled tissue before mentioned, have cellulose walls (blue with chlor-zinc-iodine), with narrow pits.

3. The cells of the **chlorophyll-parenchyma** are

thin-walled, and of oblong form: the chlorophyll-corpuscles are numerous and clearly marked.

4. The remnants of disorganized cells along the margins of the intercellular cavities, which show that they are of **lysigenetic** origin: the same may be observed with regard to the central cavity.

Add a little caustic potash to the sections mounted in glycerine, and then observe the cells of the **endodermis** under a high power: their radial walls will be seen to show the characteristic dark dot-like appearance. Passing on to the **vascular bundles,** their most marked constituents will be two to four groups of dark-looking elements, which are **tracheides of the xylem,** and are disposed, roughly speaking, in the form of a V, while the apex of the V is occupied in each bundle by a large **air-cavity.** There are originally four groups of xylem elements in each bundle, two bordering on the cavity, and two nearer the endodermis; the elements of the former are often only imperfectly seen in transverse sections, since they are apt to become disorganized during development. Between the air-cavity and the endodermis lies a mass of tissue of the **phloem,** with relatively thin cellulose walls: **sieve-plates** may sometimes be observed in surface view in this tissue.

V. Compare transverse sections of the underground axis, or root-stock, with those of the aërial axis: the sections may be prepared in the same way as the above. Note that—

1. The superficial cells have brown walls, and often grow out as long, brown, root-hairs: there are no stomata.

2. The subjacent cortex is thin-walled, and colourless, and often contains much starch.

3. The ridges, intercellular cavities of the cortex, and vascular bundles, have the same relative positions as in the aërial stem.

4. The structure of the vascular bundles is similar to that in the aërial axis.

5. There is no cavity in the centre of the axis.

Cut transverse sections of one of the tubers; treat with potash, and mount in glycerine. Observe—

1. The brown-walled epidermis with many hairs, similar to those on the root-stock.

2. A sub-epidermal layer with thickened walls.

3. The bulky parenchyma with numerous starch-grains: there are no large intercellular cavities as in the aërial axis.

4. Isolated vascular bundles, each of which is surrounded by a special endodermis, quite distinct from that of the other bundles: there is no general endodermis as in the normal axes.

A comparison of the stems of various species of *Equisetum* in respect of the endodermis shows that there is some want of uniformity in its arrangement, even in the normal axes: further, it has been shown that such differences may occur between the rhizome and the aërial axis even in the same species. In *E. arvense* there is a difference in this respect between the tubers and other axes.

VI. Make preparations suitable for the study of the epidermis in surface view, by cutting longitudinal tangential sections, and treat as before, mounting with the outer surface uppermost: observe under a high power—

1. That the superficial cells covering the ridges are of elongated form, with smooth outer walls, and

thickened, pitted, inner walls: there are no stomata on the ridges.

2. That the superficial cells of the grooves are shorter, and nearly square, their outer walls bearing those rounded excrescences already observed in transverse sections, while their whole surface is dotted with small projections: in this part are also numerous **stomata,** which present the characteristic appearance of two concentric circles, the outer being the limit of the **two subsidiary cells,** the inner that of the **two guard-cells.** Note also the peculiar radiate marking, which is due to irregularity of thickening of the wall separating the guard-cells from the subsidiary cells.

Treat sections similar to the above with Schulze's macerating fluid (see Appendix A) for some hours, and then dry them with blotting-paper, and ignite them in a spirit-lamp on platinum foil, or on a cover-glass; then treat the ash with weak acetic acid: mount the residue, and examine under a high power: a skeleton will then be found to remain, which represents clearly the several details of structure of the epidermis above described. From the treatment which the preparation has undergone it may be concluded that this is a **skeleton of silica.** (See p. 90.)

VII. Cut radial longitudinal sections of an internode of an underground stem: wash them well with water to remove as much as possible of the starch, and mount some of them in glycerine, others in chlor-zinc-iodine. Note successively the following tissues:—

1. The oblong superficial cells with brown walls, frequently bearing unicellular hairs.

2. The oblong cells of the **cortex** with cellulose walls, and containing starch.

3. The **vascular bundles,** which may be easily

recognized as transparent bands of tissue, in which may be clearly seen—

a. The elongated **tracheides** of the xylem, showing **annular, spiral,** or irregularly **reticulate** thickening of the walls: these thickenings stain yellow with chlor-zinc-iodine: there are no protoplasmic contents: the lignified rings are often found free in the intercellular cavities, owing to the rupture of the thinner parts of the walls: for this reason also the annular vessels, which adjoin the intercellular cavities in the bundles, are frequently not to be found in transverse sections.

b. The **phloem,** consisting of—

α. **Sieve-tubes,** which are elongated elements, with cellulose walls, and granular protoplasmic contents, and are divided into joints by transverse or oblique walls: they correspond in general characters to the sieve-tubes of the higher plants, but the sieve-structure of the terminal walls is not clear. Numerous highly refractive granules are found on both sides of the terminal walls.

β. **Cambiform cells** of oblong form, with cellulose walls.

VIII. From buds which have been hardened in alcohol cut median longitudinal sections: treat them for a short time with a strong solution of caustic potash, then wash them with water, and mount in strong acetic acid: or the sections may be treated with "eau de javelle" and be mounted in glycerine.

Examine them first with a low power, and observe that the **nodes** and **internodes** are easily recognized in the lower, older parts of the sections; the former

being the points of insertion of the leaf-sheaths, opposite which are various complications of the arrangement of the tissues as has already been observed: in the internodes the tissues show greater regularity of arrangement. Note that on passing towards the apex the internodes are successively shorter, and the character of the tissues of both nodes and internodes becomes more uniform: also that the leaf-sheaths become successively shorter. Following the axis upwards it may be seen to terminate in a sharp cone, which is the *punctum vegetationis*, consisting of cells undergoing division, which constitute the **primary meristem.** Here and there it may be seen that **lateral buds** have been cut through: they are situated at the nodes, and appear to be completely surrounded by the tissues at the bases of the leaves: in their form and structure they resemble the *punctum vegetationis* of the main axis, but on a smaller scale. Note also the irregularly annular or spiral tracheides in the internodes, and the way in which their structure is modified at the nodes, where they appear shorter, and are more closely reticulated.

Examine the *punctum vegetationis* under a high power, and observe—

1. At the extreme apex, a single, large, wedge-shaped cell: this is the **apical cell.** The cells immediately adjoining it are arranged in regular order, and are of definite form, being **segments** successively cut off from the apical cell. Observe how the older segments, which are further from the apical cell, have been successively divided up by walls perpendicular to the outer surface (**anticlinal**), and parallel to the outer surface

(**periclinal**). The details of arrangement of the successive walls may with advantage be traced by comparison of several preparations, and explained by reference to Figs. 13 and 14. Since the superficial cells are subject to repeated periclinal divisions, it is clear that there is no definite layer of dermatogen: compare this structure of the *punctum vegetationis* with that of the lateral buds above mentioned.

2. Note the **leaf-sheaths,** successively smaller towards the extreme apex, and observe how they originate by outgrowth and division of successive zones of cells below the apex.

3. Attention should also be paid to the mode of **origin of the lateral buds:** a diligent comparison of them in various stages of development will show that they are not of endogenous origin, but are derived from superficial cells lying immediately above the insertions of the leaf-sheaths. These cells divide, and form the young buds, which subsequently appear to be completely overarched by, and embedded in, the tissue of the leaf-sheath, and ultimately burst through it.

4. It will be useful further to trace the development of the several tissues, and to note their relations to the apical cell and its segments.

IX. Cut a series of transverse sections through a bud: prepare and mount them as above directed, being careful to keep them in their proper order of succession, and with their upper side uppermost.

Some of the sections will only have passed through the upper parts of the leaf-sheaths, which will appear as concentric rings, with a structure similar to that already observed (III.): note that the leaves of successive whorls

alternate one with another. In the centre of these rings there will be found in each of the lower sections of the series a transverse section of the axis, and one of the sections should include the *punctum vegetationis*, which would thus be seen from above. In this preparation observe that the **apical cell** appears of **triangular outline** (compare Fig. 13), while the segments are arranged regularly around it: from this observation, and from its appearance in the longitudinal section, it may be concluded that the apical cell has the form of a **three-sided pyramid,** and that segments are cut off from three sides. From the observation of transverse sections cutting the axis below the apical cell, and a comparison of these results with those drawn from a study of longitudinal sections, the mode of subdivision of the segments should be fully made out.

X. Cut transverse sections of a well-developed root of *E. arvense:* treat them with potash, and mount in glycerine: examine them under a high power, and observe—

1. That there is a peripheral band of tissue with dark brown walls: single superficial cells have grown out as root-hairs.

2. Then follows a broad band of colourless **cortex,** with large intercellular spaces: this is limited internally by—

3. A definite layer of cells having the well-marked characteristics of the **endodermis.**

4. Within this is the **pericycle,** the cells of which are opposite to those of the endodermis, and are derived with the latter from the inmost layer of the cortex. This surrounds—

5. The vascular cylinder, consisting of—

a. Four **xylem** groups, each of which may consist of only one tracheide, while one large element often occupies a central position.

b. The space intervening in each bundle between these four groups of xylem is occupied by an ill-defined group of **phloem,** and **conjunctive parenchyma.**

The arrangement of tissues at the apex of the root of *Equisetum* may be studied in the same way as above described for the root of *Aspidium Filix-mas*, and it will be found to be similar to it in all the more important points. Attention should also be paid to the mode of origin of the lateral roots, which here spring from the pericycle, while in Ferns they arise from cells of the endodermis.

The Sporangia

XI. Examine with the naked eye one of the pale-coloured, fertile stems, which rise above ground before the green vegetative shoots in the spring: observe that the internodes and leaf-sheaths of the lower part of it are similar to those of the vegetative axes. Passing upwards, note that the last leaf-sheath below the spike is of smaller size than the rest. The spike itself is covered by closely-arranged peltate scales, of hexagonal outline as seen from without: these are arranged in more or less regular whorls.

Remove some of the scales, and examine one of them in detail: it consists of a thin pedicel by which it is attached to the axis; the pedicel widens out towards its apex into a flattened shield-like structure, from the lower surface of which a number of sacs (**sporangia**) are suspended.

XII. Cut transverse sections through a spike, so as to include some of the scales: mount in glycerine, and observe under a low power. There will be seen a bulky **pith,** a ring of **vascular bundles,** and a band of cortex. The **pedicels** will appear extending radially from the axis, and widening at the outer limit into the peltate expansion, on the lower surface of which two sac-like **sporangia** may be seen.

Note that a vascular bundle runs up the pedicel, and ramifies in the peltate expansion.

Examine one of the sporangia under a high power, and note—

a. The **wall,** which is one layer of cells in thickness: the walls of these cells are strengthened by a spiral or annular thickening: the wall ruptures by a longitudinal slit on the side next the pedicel.

b. Many **spores** may be found in the sporangia, or scattered through the glycerine: examine them carefully, and observe the spirally-coiled **elaters,** and the smooth inner coats of the spore, which inclose a protoplasmic body with a well-marked nucleus.

Scatter fresh spores upon a slide, and breathe upon them gently: then observe them under the microscope: the elaters will be seen to execute active movements, thus showing that they are highly sensitive to changes of moisture in the air.

By cutting transverse sections of spikes of various stages of development, which have been hardened in alcohol, or in picric acid and then in alcohol, mounting them in glycerine, and comparing them, the history of the development of the sporangium may be traced. The chief points to be observed will be (1) that the sporangia appear as **multicellular** protuberances; (2) that a

single hypodermal cell, the **archesporium**, gives rise by division to the spore-mother-cells, while the superficial layer of cells which covers the archesporium divides into three, of which the outermost alone remains as the wall of the mature sporangium; (3) that each of the spore-mother-cells divides into **four** cells, which develop further into mature spores.

THE GAMETOPHYTE, OR OOPHYTE

The fresh spores may be sown on moist soil, and the first stages of germination, which are rapid, may be easily observed; the later stages are, however, slow, and to see these the cultures must be carefully kept. The result is the formation of prothalli (**oophoytes**) of irregular form, some of which produce **antheridia** after five to six weeks. Other prothalli, of larger size and more complicated form, produce **archegonia** after about two to three months. The antheridia are embedded in the tissue of the prothallus, and produce large spermatozoids. The archegonia are borne on the upper surface, and correspond in structure to those of the Ferns. The result of fertilization of the ovum of the archegonium is the formation of an embryo, which develops as the spore-bearing plant or **sporophyte**.

Endeavours should be made to obtain healthy cultures of the prothalli of *Equisetum*, in which the above and other points may be observed.

BRYOPHYTA

A.—MUSCI

POLYTRICHUM COMMUNE, *L*

A.—GENERAL EXTERNAL CHARACTERS

I. Observe in well-grown specimens of this Moss taken in spring or early summer—

1. The erect **stem,**[1] which may attain a considerable length, branching but rarely:

2. The **leaves,**[1] of relatively small size, and simple form; their arrangement is on a complicated plan (see p. 344): at the base of the stem note—

3. A dense mat of **rhizoids** of brownish colour.

At the apex of some specimens will be found merely a bud, composed of young leaves of the vegetative type; other specimens will bear at their apex—

4. **Perigonia,** or **perichætia;** cup-like rosettes of leaves, which assume a bright reddish or orange colour, and protect the **antheridia;** other specimens again may bear at their apex—

[1] Though the terms "stem" and "leaf" are used here, it must be distinctly borne in mind that the members thus named, being parts of the **oophyte generation** are **not homologous** with, but at most **only analogous** to the stem and leaf in vascular plants, which are parts of the **sporophyte generation.**

5. The mature **sporogonium** or spore-capsule, of which the head or **theca** is supported on a long **stalk**, or **seta.** Note in specimens which are not too ripe—

a. The **calyptra,** a dry fibrous hood, covering the apex of the sporogonium: beneath this is—

b. The lid-like **operculum** with its terminal beak: this lid may be easily detached, disclosing—

c. The pale-grey **epiphragm,** which appears as a transverse membrane, attached at its margin to the capsule by a number of short teeth of the **peristome.**

d. At the base of the theca observe a swelling called the **apophysis.**

e. By carefully removing the leaves from the apex of a plant which bears a sporogonium, it may be seen that the base of the seta is enveloped by a closely fitting sheath, the veil or **vaginula,** the origin of which will be explained later (page 350).

Observation of the external characters of a simpler Moss, *e.g. Funaria hygrometrica*, will give in the main similar results to those above described for the larger *Polytrichum.*

B.—MICROSCOPIC INVESTIGATION

Oophyte Generation

II. Cut transverse sections of a mature stem of *Polytrichum*: mount some in glycerine, others in chlor-zinc-iodine or in iodine solution: examine them first under a low power, and observe in those mounted in glycerine—

1. The outline of the section, which is usually more or less clearly triangular.

2. The dense reddish-brown band of peripheral **sclerenchyma,** which passes over gradually into—

3. A broad, thinner-walled band of tissue, which may be termed the **cortex**: this finally surrounds—

4. A central mass of firm, yellow-walled tissue.

Examine these several tissues in detail, under a high power, and observe that—

1. At the extreme outer limit is a thin **cuticle,** with small and irregular outgrowths: there is no clearly defined epidermal layer.

2. The peripheral sclerenchyma consists of cells with clearly stratified red walls, which are of such thickness as almost to obliterate the cell-cavity.

3. The broad band of tissue of the cortex has relatively thin, yellowish or colourless walls, and protoplasmic contents with starch-granules, and globules of oil (compare sections treated with iodine).

4. The central mass of tissue (which may be compared to a **vascular bundle**) is not sharply limited from the cortex: it consists of—

a. A peripheral, small-celled, and thin-walled portion, the walls of which do not stain blue, but light yellow with Schulze's solution.

b. A central, thick-walled part, without cell-contents: the thick walls stain dark brown with Schulze's solution: the elements are often divided by delicate septa, which are not stained by Schulze's solution. This tissue may be compared to the **xylem** of true vascular plants.

Here and there small groups of tissues similar to the above may be seen in the cortex; these are the strands

which enter the stem from the leaves, and pass inwards towards the central bundle.

III. Cut median longitudinal sections of the mature stem of *Polytrichum*: mount as before, and note that the peripheral sclerenchyma consists of elongated prosenchymatous elements, while the cells of the massive cortex are of a parenchymatous form. The elements of the central strand are elongated, and their lateral walls smooth, without pits: they are separated one from another by thin oblique septa. Observe that where the median plane of a leaf has been cut through longitudinally, a strand of tissues similar to those constituting the central strand may be traced, passing obliquely through the cortex towards the central strand.

By cutting transverse and longitudinal sections of the apex of the stem of *Polytrichum*, or *Funaria*, it may be ascertained that there is in each case **a single apical cell** of tetrahedral form, that segments are cut off successively from the three sides, and that one leaf originates from each segment: in the transverse sections it may, however, be seen that in both plants the angle of divergence between the successive leaves (and similarly between the successive segments) is larger than one-third: thus the leaves form three parastichies, and this will account for the apparent complexity of their arrangement in these plants. In *Fissidens* the arrangement is in two longitudinal rows or orthostichies, and there is a bilateral wedge-shaped apical cell. In *Fontinalis* there is a tetrahedral apical cell, but the divergence of both segments and leaves is one-third.

IV. Strip off a few mature leaves: mount one of them in water, with the upper surface uppermost, and observe under a low power that the narrow, linear upper portion is marked on its upper surface by longitudinal striæ (the **lamellæ**), and has a minutely

serrated margin: the basal portion of the leaf, which is closely applied to the stem, is broad, but thin and membranous, and is not marked by longitudinal striæ.

V. Cut transverse sections of leaves: this may easily be done by holding the terminal bud of a mature plant between pieces of pith, or by embedding in paraffin, and then cutting transverse sections of the whole bud. Mount all the sections as before, and examine first with a low power. Neglecting the almost circular transverse sections of the stem, recognize—

1. Those transverse sections which have passed through the sheathing basal portions of the leaves: these may be readily distinguished by their broad lateral wings, only one layer of cells in thickness.

2. Those which have been taken from the upper part of the leaf: these may be distinguished by their more bulky appearance.

Having recognized these sections, put on a high power and examine them in detail:—

1. In the section of the sheathing base of the leaf observe—

a. The two lateral wings, consisting of a single layer of cells, with thickened outer walls, and but little chlorophyll.

b. The more bulky central portion consisting of—

i. An irregular layer of superficial cells with thickened outer walls, covering both upper and lower surface: beneath these are—

ii. Bands of **sclerenchyma,** in which the lumen is almost obliterated.

iii. Within these lies a "vascular bundle" consisting

of elements essentially similar to those composing the central bundle of the stem.

2. In the sections of the upper part of the leaf note that the arrangement of the tissues is for the most part similar to that in the above sections, but rather more bulky, while opposite each of the cells at the upper surface is seen to be attached a series of three to six chlorophyll-containing cells, which represent transverse sections of those longitudinal plates or **lamellæ** above observed on the upper surface of the leaf, under a low power; the uppermost cell in each lamella (as seen in section) is enlarged and forked. It is obvious that these chlorophyll-containing lamellæ are separate laterally from one another: they constitute the chief assimilating tissue of the plant.

For comparison with the above, a Moss of simpler type may be taken, *e. g. Funaria hygrometrica.* As before there is an erect stem, with a mat of brown rhizoids at its base: it bears a number of leaves of somewhat variable form, more or less widely ovato-lanceolate.

Mount a single leaf in water and examine under a low power: note the clearly marked midrib, terminating in the acuminate apex, the thin lateral portions, consisting of only a single layer of cells containing chlorophyll, and bounded by an entire margin.

Cut transverse sections of the stem, and mount in weak glycerine or glycerine jelly: on examination under a low power it will be seen that the stem is of much simpler structure than that of *Polytrichum:* the peripheral tissues have brown walls, but they are not thickened to any marked degree: at the centre is a strand of thin-walled, small-celled tissue: the peripheral tissues usually contain chlorophyll.

Sexual Organs

VI. Take a mature antheridium-bearing axis of *Polytrichum*, and dissect it with needles in a watch-glass, keeping all the detached parts. Examine them carefully with a lens, and observe the following categories of organs—

1. The **perigonial leaves,** which are widened laterally into very broad membranous wings, with a clearly defined, central midrib.

2. The white, club-shaped **antheridia.**

3. The **paraphyses,** which will often be found associated with the antheridia: some of them are simply filamentous, others are more or less clearly spathulate.

VII. Cut median longitudinal sections ot a male axis: mount in weak glycerine, and with a low power recognize the several organs above described, and their relative positions: note especially the **antheridia** in the axils of the perigonial leaves. Observe under a high power the structure of a single antheridium: it consists of a short **stalk,** and a club-shaped body, composed of (i.) a **wall** a single layer of cells in thickness, and (ii.) a central mass of cells of more or less clearly cubical form: these are the **spermatocytes** or mother-cells of the **spermatozoids.**

VIII. Take fresh antheridium-bearing specimens of *Polytrichum*, after some days of dry weather (or keep them rather dry for some days, carefully preventing any access of water from above): squeeze one of them between the finger and thumb: the antheridia will

thus be easily forced from their position, and may be mounted in water. If they were properly mature, it may then be seen that on contact with water the antheridia burst, and the spermatocytes escape, aggregated in a mass. In each cell of this mass a spiral filament may be seen, in active movement: it may be seen to escape ultimately, owing to mucilaginous swelling of the wall of the mother-cell, as a free **spermatozoid** of spiral form, having two cilia.

Antheridia may be obtained on *Funaria hygrometrica* at almost any period of the year: they are borne on special branches as in *Polytrichum*, but are surrounded by a less conspicuous perigonium: these axes are usually shorter than those which bear the female organs.

IX. Take a sod of *Funaria*[1] with no sporogonia as yet visible upon it, but which bears antheridia: these will be situated at the apices of the shorter axes: many of the longer axes will appear to be terminated by ordinary vegetative leaves, and it is on these axes that the archegonia may be found.

From such buds, after hardening in alcohol, cut median longitudinal sections: if not transparent enough treat with dilute potash solution, and mount in weak

[1] Since *Funaria hygrometrica* produces sporogonia at all times of the year, and is very common, while *Polytrichum commune* is reproduced sexually only in the spring and early summer, it will be convenient in most cases to use the former in examining the archegonia. In either case, however, it is a matter of some experience and expenditure of time to get a good series of preparations illustrating the development and structure of the archegonium, and the early stages of the production of the sporogonium. Various other Mosses might be used for the purpose of this work, especially such as grow in tufts, and produce their sporogonia almost simultaneously.

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glycerine: subsequently the sections may be transferred to glycerine jelly. Examine first with a low power, when the usual arrangement of axis and leaves may be observed; between the youngest leaves an archegonium (or several) may sometimes be detected. If mature, it will be seen to be a flask-shaped organ, seated on a short massive **stalk**: it consists of—

1. An elongated **neck,** more or less contorted, composed of a single layer of cells arranged in four to six rows: these surround a central **canal,** which is filled with mucilage at the time of fertilization, but before maturity there may be seen within it a series of **canal cells.**

2. A lower, enlarged **ventral portion,** consisting of two layers of cells, which constitute the **wall,** and inclose a central space, in which may be seen the naked spherical **oosphere** or **ovum,** and above it (up to the period of maturity) the smaller **ventral canal cell.**

The process of fertilization cannot readily be observed in Mosses.

Similar sections, similarly prepared from rather older specimens, may show as the first results of fertilization that the neck of the archegonium turns brown and withers, while the wall of the ventral portion and the stalk show considerable increase in bulk, and frequent cell-divisions. Meanwhile growth and cell-division take place also in the fertilised ovum, resulting ultimately in the development of the sporogonium: the growth of the wall of the archegonium keeps pace for some time with that of the young sporogonium, completely inclosing it, but as it increases towards maturity, the wall of the archegonium is ruptured transversely about half-way up: the apical part is carried upward by the growing sporogonium, as the **calyptra** which covers

its apex, while the lower portion remains as an investment of the base of the seta, and is called the **veil** or **vaginula.**

Note especially in young sporogonia the two-sided, wedge-shaped apical cell, with segments cut off from either side.

Sporophyte Generation

The Sporogonium

X. Having noted the external characters of the sporogonium of *Polytrichum*, as above described, cut transverse sections of the mature seta: mount in glycerine or glycerine jelly. Being a cylindrical organ the transverse section is circular. Note—

1. The superficial layer of cells with a definite cuticle and thick yellow walls.

2. A band of brown **sclerenchyma,** which graduates internally into—

3. A thin-walled **parenchyma,** with large intercellular spaces, and containing chlorophyll.

4. Centrally is a strand of denser tissue without intercellular spaces.

XI. Cut median longitudinal sections of the base of the seta, which is inserted on the apex of the Moss-plant or oophyte: mount as before, and note in the upper part of the seta the superficial layer, brown sclerenchyma, thin-walled parenchyma, and central strand, as above described. Following the seta down to the base, it will be seen that the cuticle and brown sclerenchyma stop short, and are replaced by thin-walled parenchyma with plentiful protoplasm; this tissue of the sporophyte is in close connection with the inner surface of the

vaginula, which belongs to the oophyte generation. With this close physiological connection of the sporophyte and oophyte in the Moss, compare the connection by means of the foot in Ferns.

Similar sections may be prepared, with similar results, from plants of *Funaria*, but there will be greater difficulty in this case, owing to the smaller size of this Moss.

XII. Passing now to the apex of the sporogonium of *Polytrichum*, remove the **calyptra**: mount it in water or weak glycerine, and examine under a low power. It consists of dry, often branched, hypha-like filaments, loosely matted together: the neck of the archegonium may often be recognized at its extreme apex.

The calyptra of *Funaria* may be treated in a similar way: here the brown neck of the archegonium is clearly seen, while the body of the calyptra consists of a continuous tissue, a single layer of cells in thickness.

XIII. It will be found convenient to take first the capsule of one of the simpler Mosses, *e.g. Funaria*, and subsequently to proceed to a more complicated example, *e.g. Polytrichum*.

After noting the oval form, and the obliquely placed **operculum,** embed capsules of *Funaria*, which have been hardened in alcohol, in paraffin: cut median longitudinal sections: mount in glycerine or in glycerine jelly, and examine first with a low power.

N.B.—It will be well to select young capsules of such age that the peristome (seen through the operculum) shall show a pale yellow tinge: later it assumes a dark yellow or orange colour, and

in such cases the spores would be almost mature: the structure of the whole capsule would accordingly be more difficult to understand than in the younger specimens.

Observe—

1. The **seta,** which widens out gradually into—

2. The **theca** or **capsule,** without any clearly marked basal swelling, or apophysis: at the apex of the **theca** observe that the section has traversed—

3. The **operculum** or dome-like lid: beneath this is—

4. The yellow or orange **peristome:** in the lower part of the capsule note—

5. The outer **wall** consisting of a clearly marked **epidermis,** and beneath it some three or four layers of thin-walled parenchyma.

6. The **air-space,** traversed obliquely by filaments of thin-walled cells, suspending a central mass consisting of—

7. The **spore-sac,** which will be seen to be composed of two or three layers of thin-walled cells.

8. A layer of **spore-mother-cells,** recognized by their dense protoplasmic contents.

9. The central mass of thin-walled tissue of the **columella,** which extends upwards into the concave operculum.

By careful observation of longitudinal sections under a high power the following points may be verified:—

1. That **stomata** occur in the epidermis towards the base of the sporogonium.

This may be confirmed by cutting tangential sections from the base of the sporogonium, in which the stomata, with their two guard-cells, may be clearly seen in surface view.

2. That immediately above the upper limit of the air-space there is a band of brick-shaped cells, elongated transversely, and with pitted walls: these are not derived from the epidermis but from the subjacent layers, and serve to connect the peristome with the outer wall of the theca. Closely above this band the tissues are again more delicate, and it is here that the **rupture of the capsule** takes place, by which the operculum is set free. Above this more delicate zone is—

3. The slightly projecting lower lip of the operculum, at which point the epidermal cells are thickened, and of peculiar form, constituting the **annulus.** Beneath the operculum, which consists of the superficial layer of epidermis together with two or three layers of thin-walled tissue, lies—

4. A layer of cells with peculiar yellow or brown thickening of the walls: this gives rise to the **peristome:** further details will be given below.

Returning to the **spore-forming layer,** it may be verified under a high power—

i. That it consists at first of a **single** layer of cells (the **archesporium**): to ascertain this for certain it may be necessary to cut sections from sporogonia of various ages, and to compare them.

ii. That the cells of this layer divide repeatedly; and ultimately, by division of each of the resulting spore-mother cells into four, the **spores** are produced.

Mount some mature spores in water: they will be seen to be of spherical form, with smooth walls, and granular, oily contents.

XIV. In order better to understand the structure and origin of the **peristome,** cut off transversely the whole

orange-coloured tip of an unripe sporogonium of *Funaria*, and mount it with the apex uppermost in weak glycerine: observe under a low power—

i. The contorted brown **teeth** of the peristome, sixteen in number: these are easily seen through—

ii. The more transparent **operculum,** which covers them: by focusing carefully downwards—

iii. The much thickened cells of the **annulus** may also be distinctly seen.

XV. Cut thin transverse sections of the operculum of a still unripe sporogonium: in those which pass immediately above the annulus, observe at the periphery—

a. The thickened **epidermis,** and beneath it two or three layers of thin-walled cells: these together with the epidermis constitute the **operculum.**

b. Beneath this is found a layer of cells with peculiar thickening bands on the inner and outer walls: these bands separate, by rupture of the thinner parts of the walls, as the **inner** and **outer peristome,** which thus consists when mature of ribbands of cell-wall, and not of complete cells.

The above points may with advantage be confirmed by observations on mature sporogonia.

For comparison with the sections above described, transverse and longitudinal sections may be cut from young sporogonia of *Funaria* of various ages, and the development of this peculiar organ may thus be traced. For details of description see the text-books; especially Goebel's *Outlines*, Eng. Ed., p. 186.

XVI. Longitudinal and transverse sections may also be made from the sporogonium of *Polytrichum commune,* which may be treated as above directed. The chief differences from *Funaria* will be as follows—

1. The clearly marked apophysis.

2. The presence of **two concentric air-spaces,** with the spore-sac between them.

3. The more bulky operculum.

4. The proportionately smaller **peristome,** consisting in this case of a series of curved cells, with thickened lateral walls: their tips are connected with—

5. The **epiphragm,** which remains as a transverse membrane after the thin-walled tissue above and below it has dried up in the course of ripening of the sporogonium.

6. The less clearly marked annulus.

From these examples it appears that the peristome may be differently constructed in various mosses: it may be added that in *Splachnum* the teeth of the peristome are not, as in *Funaria*, tatters of cell-wall, but are composed of rows of cells. In order to understand the function of the peristome, where it consists, as in *Funaria*, of a fringe of tatters, take a mature sporogonium, gently remove the operculum, and examine it under a low power upon a slide, breathing upon it occasionally: the teeth of the peristome will be seen to execute movements, which appear as a succession of jerks, owing to the rough edges of the peristome catching upon one another: the spores, which appear to be forced upwards by the drying and contraction of the tissues, may thus be flicked away to a considerable distance from the mouth of the sporogonium.

In *Polytrichum* the peristome does not act in this way: at the period of ripeness the capsule becomes inverted owing to curvature of the apex of the seta, and the spores are dusted out: these points should be observed, as also similar ones relating to other Mosses.

XVII. Scatter spores from the ripe sporogonium of *Funaria* or *Polytrichum* over moist soil, and keep them at a moderately high temperature, under a bell-glass,

for a few days. The surface of the soil will soon be seen to be overgrown by numerous fine green filaments. Having carefully removed some of these with a needle, and having washed the soil from them, mount them in water, and examine them under a high power. Note—

i. The dark-coloured **exospore,** which may be found still attached to the filaments after they have attained a considerable length.

ii. The fine filamentous **protonema** resulting from out-growth of the **endospore:** observe especially the **septa,** which are often oblique; the **branches,** usually arising immediately below a septum: the various development of these branches, either—

a. As relatively thin filaments with brown cell-walls, and no chlorophyll: these are the **rhizoids,** and they penetrate the soil.

b. As relatively thick filaments, with colourless cell-walls, and chlorophyll: these constitute the true **protonema.**

c. As solid buds, which are usually situated at the base of one of the branches such as *a* or *b*: in these solid buds of various ages may be traced the successive stages of development of the **moss-plant,** which is thus produced as a lateral bud on the protonema.

Cultures of protonema, showing all the most important characters above noted, may be obtained at any time of year by cutting fine sods of *Funaria*, inverting them under a bell-glass, and growing them in moist air and at a moderate temperature for two or three weeks. In the case of *Funaria* the protonema may under these circumstances be induced to form terminal unicellular **gemmæ**, which are easily detached from the parent protonema, and by germinating reproduce the plant in a vegetative manner.

It will also be found possible, by culture of detached leaves and portions of the stem of the Moss-plant on moist soil, and under other favourable conditions, to induce a formation of protonemal filaments by direct outgrowth of cells of those parts.

Observations should also be made on the rhizoids, and protonema of various Moss-plants, by removing them from the soil, and washing them gently with water and mounting in water: examination will show the brown underground rhizoids, with oblique septa and no chlorophyll: these may rise to the surface of the soil, and develop as a branched, green protonema: or such protonemal filaments may spring from superficial cells of the stem or leaves.

SPHAGNUM (*The Bog-Moss*)

XVIII. Take a plant of any native species of *Sphagnum*, and observe with the naked eye, or with a pocket lens—

1. The brown **stem** on which are inserted—

2. **Leaves** of simple form.

3. The **lateral branches**, with fasciculate branches of higher order, which in their turn bear leaves: these branches assume two distinct forms—

a. Stronger branches, of larger size, which have their apices directed upwards.

b. Weaker branches, which are deflexed, and are usually found in close apposition to the main axis.

N.B. Owing to the main axis being thus closely covered by the weaker deflexed branches, the leaves borne by the main axis may escape observation: to prevent this, the branches should be entirely removed, and the leaves will then be easily seen in their normal position: it may further be noted on observing the leaves carefully that the lateral branches are not axillary, but are inserted **alongside of a leaf**, and that a fascicle of branches is associated thus with **every fourth leaf** of the main axis.

Nothing comparable to the protonema of other Mosses is to be found in *Sphagnum*, except under certain conditions of germination of the spore.

XIX. Cut transverse sections of the main axis of a plant of *Sphagnum*, and mount in weak glycerine or glycerine jelly: examine under a low power, and observe—

1. At the periphery of the section two or three irregular layers of cells with thin walls, and no cell-contents: lying internally to these is—

2. A dense brown band of tissue which merges gradually into—

3. A massive central pith, of comparatively large cells, with thin cell-walls and but little protoplasm.

Examine the peripheral tissues under a high power: round holes will be observed in the cells-walls as seen in surface view, while accurate observation at the points where the longitudinal walls have been cut through will show that these circular markings are actually **open pores**, by means of which the cavities of the cells of this tissue communicate one with another, and are ultimately in open communication with the medium in which the plant grows. This tissue serves as a capillary system, by means of which water is supplied to the inner tissues of the stem.

XX. Strip off one or two leaves: mount in water, or very weak glycerine, and examine under a low power: note—

1. The simple form of the leaf, and its entire margin.

2. The absence of any midrib.

3. The simple structure, the leaf consisting only of a single layer of cells, amongst which two types may be recognized under a high power, viz.—

a. Large colourless cells, the walls of which are marked by annular or spiral bands, and showing here and there round open pores similar to those already observed in the superficial layers of the stem: these cells have no cell-contents.

b. Smaller cells of narrow form, easily recognized by their containing green chlorophyll granules embedded in colourless protoplasm: these cells, being attached by their ends one to another, together form a network, the meshes of which are occupied by the large colourless cells, *a*.

XXI. It may be found difficult to obtain material for the practical study of the sexual organs of *Sphagnum*, and it will accordingly suffice here to refer to the descriptions given of them in text-books.

If specimens of the sporogonia be at hand, note with the naked eye—

1. The spherical form of the sporogonium.

2. Its position, seated on the end of a more or less elongated stalk—the **pseudopodium**: this must not be confounded with the seta of other Mosses; as sections will show, the true seta in *Sphagnum* is short, and the pseudopodium is merely a prolongation of the axis of the oophyte generation.

3. The **calyptra**, which may be, according to age, a more or less complete covering of the sporogonium, and is derived from the wall of the archegonium.

4. Where the calyptra has been broken away or removed, the circular lid-like **operculum** may be seen at the apex.

Cut longitudinal sections of the sporogonium: mount in glycerine or glycerine-jelly, and, selecting those which are median, examine under a low power—

1. The outline of the **sporogonium**, with its rounded head and short **seta**, enlarged at its base into a broad foot.

2. The **pseudopodium**, which is in close physiological connection with the foot of the sporogonium, and is continuous upwards into—

3. The **calyptra**, which may be seen, according to age, more or less completely enveloping the sporogonium.

Turning to the internal structure of the sporogonium, observe—

1. The central, massive **columella**, consisting of thin-walled cells: it is rounded off at its apex.

2. The **spore-forming layer**, which appears semilunar in section, being in reality of a bell-shape: according to the age of the sporogonium, spores of various stages of development may be seen composing the spore-forming layer.

3. Outside this is the **wall** of the sporogonium, consisting of some three or four layers of thin-walled cells, enveloped by a single layer of cells with thick brown walls. In this layer note towards the apex two points where the cells are smaller: here the section has traversed the circular line of rupture of the operculum. There is no peristome.

B.—HEPATICÆ

MARCHANTIA POLYMORPHA, *L*

A.—GENERAL EXTERNAL CHARACTERS

I. Taking a fresh growing sod of *Marchantia*, observe the following external characters with the naked eye, or by help of a pocket lens :—

1. The **flattened form,** sinuous margin, and prostrate position of the branched, green thallus.

2. Its dull, dark green upper surface, marked by diamond-shaped areas, and in the middle of each of these a dot, which is a single **stoma** (see below).

3. Projecting from the upper surface may also be seen in most cases small **circular cups,** with a finely crenate margin, in which may be seen numerous dark green flattened bodies, the **gemmæ :** these may be easily detached by slight mechanical disturbance.

4. Note the **organic apex** of the thallus, situated at the base of a terminal depression (compare the prothallus of Ferns) : also that the branching is **dichotomous,** though the ultimate development of the originally similar branches is unequal, so that the result is a **sympodium.**

5. In some cases the branches of the thallus may have assumed peculiar forms, together with an **erect**

position: these are the branches which bear the sexual organs, and two different types may be easily recognized as borne upon different individual plants, viz.—

a. Branches with a relatively thin stalk, bearing a terminal disk with crenate margin, and having numerous dot-like markings on the upper surface: these are the **male branches,** having the **antheridia** on their upper surface.

b. Branches, also with thin stalks, bearing a terminal star about $\frac{1}{4}$ inch to $\frac{1}{2}$ inch in diameter: these are the **female branches,** which produce the **archegonia** on their under surface, and ultimately the **sporogonia** and **spores.**

II. Remove a thallus carefully from the soil, and wash with water, taking care not to injure it, and examine the organs on its lower surface with a pocket lens: note especially—

1. The numerous **rhizoids** or root-hairs, attached chiefly to the central midrib.

2. The **amphigastria,** white or purple lamellæ attached to the lower surface of the thallus, and most clearly seen in the regions near the apex, where they are closely aggregated so as to protect the young tissue. These may be compared with the "leaves" of the Mosses (cf. note, page 1).

There will frequently be found growing in positions suitable for *Marchantia*, and often associated with it, another Liverwort called *Lunularia*, which is similar in general contour, but is usually of a lighter green colour: it may readily be distinguished by the form of the cups containing the gemmæ, these being, as the name *Lunularia* implies, of a semilunar form. It is to be

remarked further that *Lunularia* differs from *Marchantia* in certain structural details ; and also in the fact that, though reproduction by gemmæ is most profuse, a sexual reproduction is not effected in this country. Thus it cannot be taken as a substitute for *Marchantia*, and the material must be carefully sorted before use.

For comparison with *Marchantia*, observations should also be made of other allied forms, *e.g. Riccia fluitans*, which is of simpler structure, and of much smaller size ; it branches dichotomously, and the branches in this case often develop equally. If a fresh thallus of *Riccia* be examined under a low power, it will be seen to consist of undifferentiated green chlorophyll-containing tissue, and the apex will be seen to be depressed ; on the under surface will be found small amphigastria.

As an alternative or additional type *Pellia epiphylla* may be taken : it is a thalloid Liverwort, very commonly to be found growing on moist banks, and by streams. It appears as a flat expanded green thallus, with smooth upper surface, sinuous margin, and slightly thickened midrib. The thallus is frequently branched in a dichotomous manner, but the development of the branch-system is sympodial : numerous root-hairs spring from the lower surface of the midrib : amphigastria are absent.

Some specimens may be found in spring to be dotted over the upper surface, and especially about the midrib, by numerous roundish bodies, partly sunk in the tissue of the thallus—these are the antheridia. Here and there, on the upper surface of the same or of different specimens, pocket-like cavities will be found at the apex of branches of the thallus, while in spring a round-headed, stalked body—the sporogonium—may be seen projecting from each. When ripe the round head

of the sporogonium is raised upon the top of the greatly elongated and transparent seta.

Some species of *Jungermannia* should also be examined as an example of the foliose Liverworts: here the shoot will be found to be dorsiventral, but of more complex external form than that of *Marchantia;* in addition to the amphigastria, there are also present lateral appendages or "leaves," which are thin, flattened expansions of green chlorophyll-containing tissue, inserted on the relatively thin axis.

B.—MICROSCOPIC OBSERVATIONS

III. Cut transverse sections of the vegetative thallus of *Marchantia,* avoiding at first the cups bearing the gemmæ, and the sexual branches. It is easier to use material hardened in alcohol, and to embed it in paraffin, or hold it between pieces of pith; but if sections be cut from fresh green material the presence of chlorophyll will be found to be an advantage in distinguishing the tissues. Mount some sections in weak glycerine, others in chlor-zinc-iodine, or in iodine solution: examine under a high power, and, starting from the upper surface, observe—

a. The **superficial layer,** or so-called "epidermis," consisting of a continuous layer of cells of small size which contain chlorophyll, as may be seen in preparations from fresh material: the continuity of the layer is broken here and there by the so-called "stomata": these, however, differ from the true stomata of the higher plants in the mode of their development. This layer may be seen to be attached to the lower-lying tissues at points between the "stomata," and the lines of attach-

ment thus cut through correspond to the limits of the diamond-shaped areas above noted.

b. Beneath the "stomata" are large areas, the **air-cavities,** in which are seen numerous round or oval cells, grouped in simple or branched series, and attached to the lower surface of the cavity: their cell-walls are thin, and consist of cellulose: these cells contain chlorophyll, and constitute the chief assimilating tissue of the plant.

c. Below this is a massive tissue, which constitutes the great bulk of the section: it consists of oval cells, with few intercellular spaces, if any at all: the walls are thin, and marked with shallow pits: the protoplasmic contents are scanty: in the cells nearer the upper surface there are often numerous starch-grains. Individual cells here and there in this tissue have peculiar mucilaginous, or highly refractive, yellowish or brown, oily contents.

Cut transverse sections from a fresh thallus, mount in water, and having noted cells containing the highly refractive bodies above mentioned, treat with potash solution, and warm gently over a spirit lamp: the bodies are not dissolved, but partially lose their highly refractive quality, without swelling. Treat other sections first with alcohol, and then with ether: wash from the ether with alcohol, and mount: the oil bodies will have been dissolved.

d. At the lower surface of the thallus may be seen attached organs of two kinds—

i. **Hairs,** or **rhizoids,** which are long and unicellular, and are inserted deeply in the tissues of the thallus: they often show dotted or peg-like ingrowths of the cell-wall of various form.

ii. The **amphigastria,** which may now be seen to be plates of tissue one layer of cells in thickness: their cell-walls are often coloured violet or brown.

Returning now to the "**stomata,**" note under a high power their structure as seen in a good transverse section: each will appear as consisting of tiers of small cells (four or more in depth), which surround a large central cavity.

IV. Cut tangential sections so as to strip off the so-called "**epidermis**": mount with the outer surface uppermost in weak glycerine: observe under a low power the diamond-shaped areas above described, and a single large "stoma" in the middle of each. Under a high power note—

1. That the cells of the "epidermis" contain chlorophyll.

2. That each "stoma" is bounded by four or five of the tiers of cells above described.

3. That these cells contain but little chlorophyll.

4. That on focusing downwards it becomes apparent that the lowest cell of each tier projects into the cavity of the "stoma," so that the channel at that point presents a stellate appearance in surface view.

Sections should also be cut from the thallus of *Lunularia*, wherein it will be found that the stomata do not correspond to the above description, but are of a simpler type, and it is well to compare the structure in *Lunularia* with that in *Marchantia*.

Cut longitudinal sections of a mature thallus of *Pellia epiphylla*: if the material be fresh, mount in water, if hardened in alcohol mount in glycerine, and observe the almost uniform structure of the tissues: the chlorophyll is chiefly located at the upper surface; the lowest layer of cells is coloured brown, and

gives rise to numerous unicellular rhizoids, but there are no amphigastria.

Note especially that the internal cells have their otherwise thin walls strengthened by vertically running brown bars: where cut through, these are seen to be the result of an equal thickening of the walls on both sides.

Cut also transverse sections, and compare them: these demonstrate—

1. That the thallus which is massive in the middle, thins out at the margins to a single layer of cells.

2. That the thickening bars form networks in vertical planes, and only occasionally extend in a longitudinal direction.

V. Remove a number of the **rhizoids**: mount them in water or glycerine, and examine under a low power: two types of these elongated unbranched hairs may be recognized—

a. Those with smooth walls: these are the more numerous:

b. Those with peg-like projections of the wall into the cavity of the hair: these projections are arranged in a more or less clearly spiral manner, and they may not unfrequently be seen to be branched irregularly: the protoplasmic contents are very scanty.

VI. Avoiding, as before, both the cups with gemmæ, and the sexual branches, cut from material hardened in spirit fine median longitudinal sections of the thallus, so as to include the depressed apex: mount in glycerine, and examine under a high power.

In the older part of the section the structure of the thallus will be seen as above described: on approaching the apex, note that the air-cavities are successively smaller, and the chlorophyll-containing tissue gradually disappears. The thallus has a **blunt apex** covered by numerous amphigastria, which thus protect it. At the extreme organic apex may be seen a single wedge-shaped

cell, which is, however, only one of a **series of initial cells** of like form, as may be ascertained on careful investigation by means of transverse sections of the apex. In good median longitudinal sections of the apex, note also—

1. The origin of the amphigastria, as plate-like outgrowths on the ventral surface, immediately below the apex of the thallus.

2. The formation of the air-cavities: these appear to be formed by involution of the outer surface, and subsequent over-arching of the cavity thus formed; but the actual proof of this is a matter of some difficulty, and requires careful cutting of sections.

3. The development of the so-called "stomata" by division of certain cells by periclinal walls.

4. The origin of the chlorophyll-containing tissue, by budding of the cells forming the floor of the young air-cavity, and frequent subsequent branching.

Similar preparations may be made from *Lunularia* and *Fegatella* with similar results: in these it is more easy to trace the origin of the air-cavities. Note especially in these forms the simpler structure of the "stomata" and the peculiar mucilage-cells, which in *Fegatella* are associated in longitudinal series, and attain a large size: the usual tests for mucilage should be applied (see p. 94).

Preparations should also be made to illustrate the structure of the apical meristem in other forms: *Metzgeria furcata* will be found to be an excellent object for this purpose. It is commonly found growing on damp rocks, tree trunks, &c., especially in mountain districts. In order to see the single two-sided, wedge-shaped apical cell, fresh or hardened material is to be treated till quite transparent with "eau de javelle," and mounted in glycerine: in apices thus treated the apical cell and its segmentation, as well as the dichotomous mode of branching of the thallus may be readily seen. In *Jungermannia* there is a three-sided, and in *Blasia* a four-sided apical cell. These examples will suffice to show that the type of apical structure is far from being uniform throughout the Liverworts.

Gemmæ.

VII. Remove from one of the cups on the upper surface of the fresh thallus of *Marchantia* some of the **gemmæ**: mount them in water, and note under a low power—

1. The flattened disk-like form of the gemma, presenting a nearly circular outline, with two lateral indentations, and a scar at the base where it was attached to the thallus which produced it.

2. The ordinary chlorophyll-parenchyma of which it is mainly composed.

3. Superficial hyaline cells, from which the rhizoids are subsequently derived.

4. The single cells containing oil-bodies.

It may further be observed that the gemma is in its peripheral part only one layer of cells in thickness, while the central part is a solid mass: also that the structure is alike on both sides of the gemma, *i.e.* that it does not as yet show any trace of a dorsiventral character.

VIII. Cut transverse sections of a thallus so as to pass through the middle of one of the **cups**: mount in very weak glycerine, or in water, and examine under a low power: note—

1. The two lips of the cup, which appear as outgrowths from the upper surface of the thallus, and show more or less clearly the same structure, especially in the lower part.

2. The numerous **gemmæ**, in various stages of development, which are attached to the base of the cup by unicellular stalks.

3. Unicellular outgrowths or hairs, with mucilaginous walls.

Trace under a high power the process of development of the gemma. It may be seen that the gemma originates as a unicellular papilla, which divides first into two cells: the lower remains quiescent as the unicellular stalk, while the upper cell grows and divides, ultimately giving rise to the mature gemma. Trace the succession of cell-divisions by comparison of gemmæ of various ages.

Removing some gemmæ from the cups, germinate them on clean moist sand under a bell-glass at a medium temperature, and exposed to the light: on examining them after five or six days, they will be seen to be elongated transversely to their original axis of growth, the base of each of the lateral indentations serving as an organic apex: from the lower surface root-hairs have been formed, by simple outgrowth of single cells. After growth has been continued for a longer time the differentiation of tissues characteristic of the mature thallus, with "stomata" and air-cavities, becomes apparent on the upper surface, the thallus thus assuming a dorsiventral character.

No gemmæ are produced in *Pellia*.

The Male Branch (*Antheridiophore*)

IX. Having noted the general form of the male branch of the thallus, with its stalk and terminal disk, cut transverse sections of the stalk, mount in glycerine, and examine under a medium power. Observe—

1. The circular **outline** of the sections.

2. Two **involutions** of the margin, corresponding to two channels, which traverse the stalk longitudinally: due investigation will show that these are on the morphologically lower face of the stalk.

3. **Rhizoids** will be found traversing these channels longitudinally.

4. The tissues seen in these sections are not clearly differentiated.

X. Cut median vertical sections through the terminal disk: mount in weak glycerine, and examine first under a low power: observe—

1. The general **outline** of the section, and especially the attachment of the disk, and the flat upper surface.

2. The **amphigastria** and **rhizoids** attached to the lower surface.

3. The **cavities** in the tissue, of two sorts, both opening by narrow mouths on the upper surface—

a. **Air-cavities** fundamentally similar to those of the vegetative thallus, with a "stoma" above the centre of each, and with chlorophyll-containing cells as before.

b. **Flask-shaped cavities** each containing one **antheridium**: these also open by a narrow channel on the upper surface of the disk.

Look for a single ripe antheridium which has been cut through longitudinally: having found one, examine it in detail under a high power, and observe—

1. The short **stalk** by which it is attached to the base of the cavity.

2. The **wall** of the antheridium, consisting of a single layer of thin-walled cells.

3. The **mother-cells of the spermatozoids** of

cubical form, and small size, which together constitute a dense central mass.

Trace the channel of one of the flask-shaped cavities up to the surface of the thallus, and note that the structure of the pore is quite distinct and different from that of the "stoma."

Compare antheridia of various ages in sections cut from younger male branches: by such a comparison it may be ascertained that each antheridium arises from a single cell at the base of the flask-like cavity: trace the successive divisions which accompany the development of one such single cell into the mature antheridium.

Cut tangential sections from the upper surface of a male branch, and under a high power note—

a. The "stomata," of similar character to those on the vegetative thallus.

b. The pores or openings of the cavities in which the antheridia are seated. These pores are of about the same size as the "stomata," but differ from them in being often triangular, and in the number (nine) and arrangement of the surrounding cells.

Cut median longitudinal sections of a thallus of *Pellia* on which antheridia are borne (see p. 362): the antheridia are mature in late spring or early summer, and will be found in various stages of development in material taken in the early part of the year. Examine the sections with a low power, and the antheridia will be readily seen, having a form and structure similar to those of *Marchantia:* each is seated singly, in a flask-like depression of the upper surface of the thallus, and covered over by a circular lip, which projects beyond the general surface of the thallus, and is traversed by a very narrow pore: through this the spermatozoids escape.

Similar sections cut through the apex of a thallus taken in spring, will show antheridia in various stages of development, and will demonstrate that, as in *Marchantia*, each originates from a single superficial cell of the upper surface of the thallus.

The Female Branch (Archegoniophore)

XI. Remove the star-shaped head of a female branch which has attained a considerable length, and examine first the upper surface: with the naked eye or with a lens note the rounded arms, usually nine in number, and the diamond-shaped areas, each having a single central stoma.

Turn the head upside down, and observe on its lower surface—

1. The central attachment or **stalk.**

2. The radiating **arms,** usually nine in number.

3. The curtain-like **perichætia,** which alternate in position with the arms: the **archegonia** are enveloped by these, and if the branch be an old one—

4. The nearly spherical **sporogonia** may be observed protruding from them: if these be fully ripe, they may have burst, in which case a yellow flocculent mass may be seen protruding from them, consisting of the **spores** and **elaters.**

XII. Cut transverse sections of the stalk, and mount in glycerine: examine under a medium power, and compare with similar sections of the stalk of the male branch: the general arrangement of the parts is the same, the chief differences being in this case—

1. The quadrangular outline of the sections.

2. The presence of air-cavities on the morphologically upper surface, with "stomata," and small quantities of chlorophyll-containing parenchyma: in fact the structure of the female stalk corresponds more nearly than the male to that of the ordinary vegetative

thallus, of which they may both be regarded as modifications.

XIII. It will be well to start the study of the female organs on a younger branch than that above described: select a female branch which has not yet grown more than a quarter of an inch in height,—cut rather thick transverse sections (*i.e.* in a horizontal plane) through the head of it, mount in glycerine, and examine under a low power: observe—

1. The central **stalk** cut through transversely, and presenting characters similar to those above described.

2. The **arms,** usually nine in number, radiating from that central point (it may be further noted that the largest gap between the arms is directly opposite the morphologically upper surface of the stalk, and that in this gap there is no group of archegonia):

3. The numerous **archegonia,** each of which presents a circular outline from this point of view: they are disposed in groups alternating in position with the arms, each group being surrounded by the perichætium.

Under a high power the following points may be ascertained—

a. That the most mature archegonia are those nearest the periphery, while those of each group are successively younger the nearer they are to the central stalk.

b. That each mature archegonium as seen in section consists of a wall (one layer of cells in thickness) which surrounds and incloses a large, naked, nucleated **ovum.**

XIV. Cut vertical sections through a female branch of like age to the above: treat as before, and observe in median sections—

1. The **stalk,** on which is borne—
2. The terminal stellate **head.**
3. That the latter consists of tissues similar to those of the vegetative thallus: thus there may be seen—
   a. The superficial "epidermis," and "stomata."
   b. Air-cavities with chlorophyll-parenchyma.
   c. Large mucilage-cells.
4. The **archegonia,** which are flask-shaped bodies suspended in an inverted position: note in a mature archegonium—
   a. The long **neck,** consisting of many tiers of cells, which together form a single-layered cylinder surrounding the channel of the neck: when mature the neck will be seen to be open at its apex.
   b. The more distended lower ventral part of the archegonium, also surrounded by a single-layered wall, and attached by a short massive stalk to the receptacle.
   c. Pay special attention to the contents of the ventral portion: in the mature archegonium the cavity will be seen to be occupied by a single primordial cell—the **ovum** or **oosphere**—to which access is gained from without through the channel of the neck.

It is perhaps easier in these plants than in any others to trace the successive stages of development of the archegonium: this may be done by cutting sections, similar to those above described from young female receptacles, and noting first those archegonia which are situated nearest to the stalk; these should then be compared with those further removed from it, which will be found to represent successively older specimens: thus the several stages of development may be traced even in a single

specimen, and they should be carefully compared with the description and figures given in the standard text-books (*e.g.* Goebel, *Outlines of Classification*, Eng. Ed. pp. 150, 162). The attempt should further be made to observe the actual rupture of the neck of the archegonium, by preparing sections of the female receptacle, mounting dry ; then, having found a suitable archegonium, add a drop of water, when the canal cells and ventral canal cell may be seen to be suddenly extruded on rupture of the apex of the neck.

Longitudinal sections should also be cut from the thallus of *Pellia* taken in spring, so as to traverse one of the young pockets, or involucres : inserted on the wide base of the pocket may be found one or more archegonia in a horizontal position : their structure is essentially similar to that in *Marchantia*. Various stages of development of archegonia may be found in the same section, or they can be obtained by comparing sections from different specimens.

XV. Keep some specimens of *Marchantia*, having mature male receptacles, protected for some days from access of water from above : then place a drop of water on the upper surface of a receptacle, and after a short time transfer it to a glass slide, and examine under a high power : there will then be seen numerous motile **spermatozoids** of elongated, slightly curved form, and they are kept in active motion by two **cilia** attached to the anterior end. In order to make them clearly visible they should be killed and stained by adding a small quantity of iodine solution, or of osmic acid.

XVI. Add a drop of water containing living spermatozoids to a fresh preparation of a female receptacle containing mature archegonia : note the directive influence of the archegonia in attracting the spermatozoids to the neck, which they enter, and are lost to sight in the mucilage which fills it.

MARCHANTIA.—SPOROPHYTE

XVII. Cut median longitudinal sections of a female receptacle bearing almost mature sporogonia: mount as before, and note under a low power that the parts of the receptacle remain as before: but observe especially—

1. The **perigynium,** a loose sac-like coat, which arises from the receptacle after fertilisation, and envelops the archegonium during its further growth.

2. The **wall** of the archegonium, now consisting of two layers of cells, and still bearing at its apex the **neck,** which shows signs of withering.

3. The **sporogonium,** an almost spherical body inclosed by the enlarged venter of the archegonium: the following parts of it are to be recognised—

a. The massive conical foot or **seta** at the end remote from the neck, *i.e.* directed towards the base of the archegonium.

b. The **wall** of the remaining portion of the sporogonium (**capsule**), consisting of a single layer of cells.

c. The hemispherical **sporogenic mass,** with no central columella: in it may be recognised (i.) the **elaters,** long spindle-shaped cells, arranged in a fan-like manner as seen in section; and (ii.) the **spores.**

Mount a small portion of the yellow flocculent mass which escapes on the rupture of a mature sporogonium, and breathe gently on it, observing it the while under a low power: note the hygroscopic movements of the

spirally thickened elaters, and the consequent separation and scattering of the spores.

Sections should also be cut through female receptacles of various ages, and by a comparison of these the development of the sporogonium may be traced from the first divisions of the fertilised ovum to the mature condition.

For comparison with those of *Marchantia* the sporogonia of *Pellia* should also be examined. Cut median longitudinal sections of a thallus of *Pellia*, so as to traverse a young sporogonium of such age, that its round head may just be seen projecting from the pocket (see p. 362) : this is the condition of the sporogonia during the winter, for those which are the result of fertilisation in the early summer of one year do not scatter their spores till the spring of the year following. In such sections observe—

1. The thallus which bears the sporogonium, while round the sporogonium may be recognised—
   - *a.* An outer protective flap on either side: this is the **involucre.**
   - *b.* The more or less complete remains of the **calyptra,** which is developed from the wall of the archegonium, and may still cover the sporogonium completely.
2. The sporogonium, consisting of—
   - *a.* The **seta,** with its enlarged triangular foot, by which it is inserted on the tissue of the thallus: the upper part of the seta is cylindrical, and is composed of rows of narrow discoid cells, with much starch : it is the enlargement of these cells which brings about the extension of the seta.
   - *b.* The **head** of the sporogonium, which consists of a wall composed of two layers : this surrounds the spherical mass of spores and elaters. Note

the structure of the cells of the wall: the outer layer have soft cell-walls, the inner firm and dark-coloured walls, an arrangement which will result in the opening of the sporogonium on drying.

The spores are not simple cells, but undergo division of the contents before they are shed.

It will be well also to compare the sporogonium of *Anthoceros*, noting the foliaceous irregularly-branched thallus, the upright, elongated, and cylindrical sporogonia, which are inserted on the upper surface of the thallus, and are surrounded at the base by a sheath. The oldest sporogonia may have begun to split into two equal halves from the apex downwards.

Cut median longitudinal sections of a sporogonium of medium age, and observe the enlarged **foot** with hair-like outgrowths penetrating the tissue of the oophyte: the **zone of basifugal intercalary growth** immediately above this: the capsule above will be seen to consist of a wall four or five layers of cells in thickness, and having stomata; a sporogenic layer, in which the division of the cells into four may be easily recognised: and a thin central **columella.** Transverse sections will show that the wall is thinner at two points than elsewhere, and it is here that the rupture takes place when the sporogonium matures.

THALLOPHYTA

FLORIDEÆ

POLYSIPHONIA FASTIGIATA, *Grev.*

I. This seaweed is found on all our coasts, growing in dense reddish-brown tufts, which are fixed firmly on to the thallus of *Ascophyllum* (*Ozothallia*) *nodosum*, Le Jolis. It grows to a length of about two inches, and the thin cylindrical thallus is frequently branched in an apparently dichotomous manner: on some of the plants taken in autumn there may be recognised with the naked eye, or with a simple lens, roundish bodies borne laterally (**cystocarps**); on others irregular yellowish tassels at the ends of the branches, these are the **antheridia,** and they are best seen on specimens taken in early summer; on others again dark irregularly disposed swellings may be recognised in the substance of the thallus, these are the organs of vegetative reproduction (**tetraspores**).

II. The material to be used for microscopic investi gation should be either quite fresh, and be kept, and mounted in salt water, or better in weak glycerine, or it should be treated while quite fresh with a solution of picric acid in salt water (see p. 6), and after washing

with sea-water, be hardened in successively stronger alcohol from 40 to 90 per cent.

From material thus treated, select a thallus which does not apparently bear any of the reproductive organs above mentioned; mount a piece of it, including the tips of some of the branches, in 50 per cent. glycerine and water, and observe under a low power—

1. The cylindrical form of the thallus, and the slight inequality of the apparently dichotomous branching.

2. The general structure of the mature parts of the thallus, consisting of—

a. A series of large **central cells,** with dark reddish-brown contents: these are surrounded by—

b. A single layer of elongated **pericentral or cortical cells,** which are arranged with considerable regularity in rings, each ring corresponding to, and surrounding one of the central cells: the whole thallus is thus built up of successive **tiers of cells.**

3. Observe also the apices of the branches, which taper off to fine points, each terminating in a single dome-shaped **apical cell.**

Select a good specimen of an apex, and examine it in detail under a high power: observe—

i. The conical ending of the branch, covered by a thick mucilaginous wall, which extends backwards over the more mature parts, and is covered externally by a definite and continuous **cuticle.**

ii. The single dome-shaped **apical cell,** with highly refractive protoplasmic contents, and more or less obvious nucleus.

iii. The successive segments which have been cut off from its base by parallel transverse walls.

iv. The subdivision of the segments by longitudinal walls, so that each segment ultimately forms one of those tiers of cells (consisting of one central and numerous pericentral cells) of which the whole thallus is built up.

Compare a number of apices in order to ascertain the mode of branching: it will be seen that in some apices the apical cell will have divided by an **oblique** instead of a transverse wall: both of the two cells thus formed gradually assume the properties of apical cells, dividing in the usual way, and growing out right and left as almost equal branches; but the inequality of origin is still to be traced even in old branchings: it is thus obvious that this is not a typical dichotomous branching, but **a form of monopodial branching** closely allied to it.

A perfectly typical example of dichotomy is to be found in *Dictyota dichotoma*, and it may be easily observed on mounting a piece of the thallus in weak glycerine, and examining under a medium power.

In mature parts of the thallus, as also near the young apex, note carefully under a high power the fine protoplasmic strands, which extend through the swollen cell-wall, connecting the protoplasmic body of the various cells of the thallus one with another: for further study of these recourse must be had to sections of the thallus.

III. Embed, and cut transverse sections of the thallus of *Polysiphonia*, selecting such a part of it as is not too old, *i.e.* about half-way between the apex and the base: mount in glycerine and examine under a high power:

it may then be seen in those sections which pass through the middle of one of the tiers of cells above noted that—

1. The section is circular, since the thallus is cylindrical.

2. That it is limited externally by a clearly marked **cuticle,** and it will be remembered that as *Ascophyllum*, on which it grows, is found about half-tide mark, the plant is exposed to the air for several hours in each tide.

3. The series of **pericentral cells,** of variable number, each surrounded by a massive, stratified cell-wall, and having a dense, highly refractive, protoplasmic body.

4. The single large **central cell,** having similar characters of wall and protoplasm to the pericentral cells.

5. Note especially the **protoplasmic strands,** which run from the central cell to the several pericentral cells, traversing the cell-wall.

The recognition of these connecting strands of protoplasm between the cells of the thallus of the Florideæ dates back to a period before "protoplasmic continuity" had acquired the special interest which it now possesses ; nevertheless, there is still some difference of opinion as to the details of their structure : some hold that, at least in some cases, the strands are continuous, without any break, from one cell to another ; that this is not the case universally is obvious, as **pit-membranes** may be seen (and clearly in the majority of cases in *P. fastigiata*) traversing the strands transversely. It has further been asserted that, where this is the case, the pit-membrane has the character of a **sieve;** but still Schmitz holds that there is no exchange of protoplasm as such, since the pit-membrane is covered on either side by

thick plates of a highly-refractive substance ("stoppers" of Wright), which are themselves, however, connected by fine strands passing through the pit-membrane.

Valuable information on various points is to be gained by treating fresh specimens of thalli, which do not bear reproductive organs, with slightly dilute sulphuric acid for about an hour: material thus prepared is to be mounted in water or weak glycerine, and by a slight pressure with a needle on the cover-slip the protoplasmic bodies of the cells may be disengaged from the greatly swollen cell-walls: they will often be seen to hang together in groups, and the fine protoplasmic strands (with a more or less obvious sieve arrangement) will then be seen extending between them: a subsequent staining with Hofmann's blue will make this more apparent. Special attention should be given to the subdivision of the successive segments cut off from the apical cell, since the pericentral cells are derived from them in a peculiar manner. It may be seen that they are formed, not simultaneously, but **successively.** The first is cut off in form of a wedge, and the rest of the pericentral cells appear alternately on either side of the first, until the whole periphery of the segment is occupied by a series of pericentral cells surrounding the central one from which they are derived.

A careful observation of young apices externally, under a high power, will be found to bear out this observation.

It is stated that the protoplasmic strands connecting the central cells together are of primary origin, being formed at the time of cell-division; but that those connecting the cortical cells of one tier with those of the next are of secondary origin.

IV. Longitudinal sections should also be cut so as to complete the study of the tissues: in median longitudinal sections the series of central cells will be obvious under a low power, the dark protoplasmic body being surrounded by a swollen and beautifully stratified cell-wall. Externally will be seen the pericentral cells of oblong form, their free walls being covered by the

continuous cuticle. Pay special attention, under a high power, to the protoplasmic strands, which connect the cells of the central series one with another, and also with the pericentral cells. The former are thick strands, with large and obvious "stoppers" on either side of the pit-membrane; the latter are much thinner, and the presence of the pit-membrane, and "stoppers" is consequently less easy to observe, if indeed they be constantly present at all.

V. Turning now to the insertion on the frond of *Ascophyllum*, detach the bases of one or two of the thalli of *P. fastigiata* from the substratum by means of forceps, noting the firmness of their attachment: mount, and examine under a low power. It will then be seen that towards the base of the thallus single superficial cells are of elongated, cylindrical form, and have thick walls.

VI. Cut transverse sections of the frond of *Ascophyllum*, so as to pass through the insertion of one of the tufts of *Polysiphonia:* mount as before, and observe under a low power that those elongated, thick-walled cells above described penetrate deeply into the thallus of the host, and thus obtain a firm hold upon it, while their own strongly thickened walls will explain further the strength of attachment.

For comparison with the above type, observations should also be made on other forms among the Florideæ, and the following are suggested as good illustrative examples:

A. *Batrachospermum moniliforme*, Roth., a purplish-brown, slimy-feeling Alga, which grows on stones in fresh-water streams. Observe it fresh, mounted in water, or preserved in weak glycerine, and note under a medium power the central series of

cells, which terminates in a dome-shaped apical cell: in the mature parts, the cells of the central series are elongated, as internodes, while at the nodes are inserted brushes of branched multicellular filaments, often terminating in long fine bristles: in the older parts, some of these filaments are closely applied to the internodal cells, thus forming a sort of cortex.

B. *Ptilota plumosa*, Ag., a small, dark-purple seaweed, found growing on vertical or overhanging rocks: mount in glycerine, or, if fresh, in sea-water. Observe a well-marked central series of cells, which are individually short, and there is a single apical cell as before: branches are inserted, in one plane, on either side of the central series: in the older portions a cortex surrounds the central cells, and it may be traced as originating partly by outgrowth and division of the central series, partly from the bases of the branches.

C. *Chondrus crispus*, Stack (Irish Moss or Carragheen), should also be observed as an example of a more massive thallus; it is very variable in outline, according to the conditions under which it grows: the branching is dichotomous, and the thallus more or less broadly strap-shaped, being attached by a disk-like organ to the substratum.

Cut transverse sections of the thallus, and mount in pure glycerine: examine under a high power, and note—

1. The superficial cuticle.

2. The outer cortex, composed of linear series of small cells.

3. The inner cortex, consisting of larger cells, with swollen cell-walls, which are traversed by protoplasmic threads: these are, however, usually (if not always) intercepted by a highly refractive pit-membrane, the small size of which makes it difficult to trace any sieve-like structure.

4. A central mass of hyphal tissue.

Thus there is no central linear series as in the plants previously described: the whole structure approaches rather to that of *Fucus* (see below).

D. *Corallina officinalis*, L., should also be examined as a type of the lime-incrusted Corallines: it is common between the tide-marks on all our coasts. Note the mode of branching of the thallus and its calcareous consistency. Sections should be cut from the

tissues, either after dissolving the lime with acetic acid, or, better, by grinding as in the preparation of geological sections: in the latter case observe—

1. The successive pear-shaped joints of lime-incrusted tissue: these can be recognized externally with the naked eye.

2. The intervening transparent zones of tissue with pliant mucilaginous walls.

The whole is made up of cells of nearly equal size, arranged in linear series, which correspond to one another in such a way that the cells appear in fan-like zones. Here again there is no single central series larger than the rest.

E. Observations may also be made with advantage on specimens of *Lemanea*, a genus of dark olive-green, fresh-water Algæ, included in the Florideæ: these plants grow attached to rocks in mountain streams. Note especially—

1. The simple filamentous, and branched proembryos similar in many respects to that of *Chara:* these are known as the "*Chantransia*" filaments, and it is by means of them that the plant is attached to the rocks.

2. The more complex "reproductive thallus" which is produced as a lateral branch upon it: in the character of the apex, and in its mature structure, it is not unlike that of some species of *Polysiphonia:* it has clearly marked nodes, around which the irregular antheridial patches are situated.

Transverse and longitudinal sections should be cut, from which it will be seen that the "reproductive thallus" consists of a central series of elongated cells, which traverses a cavity filled with mucilage, and is connected by lateral struts, at points between the nodes, with the cortical tissue: the latter consists of relatively large internal cells, and relatively small superficial ones.

VII. Having distinguished with a lens, or under a low power, a specimen bearing tetraspores, mount a portion of it as before directed (taking care that young branches as well as mature ones are represented), and examine under a medium or high power. Note—

1. That the regularity of the tissues of the thallus is

disturbed at certain points by dark spherical bodies, which lie embedded in the tissue below the pericentral cells: these are the **tetraspores.**

2. That they decrease in size as the apex of the branchlet is approached.

3. That they have no clearly definite arrangement.

4. That each undergoes a division into four, hence the term "tetraspore."

5. That they escape by rupture of the layer of pericentral cells: note in older parts the vacant cavities whence tetraspores have escaped.

6. That the mature tetraspores are naked, spherical, and motionless protoplasmic bodies.

By a careful comparison of tetraspores in early stages of development it may be ascertained that they originate from cells of the central series, as shortly-stalked bodies, of which the head enlarges and divides into four, while the pedicel remains small.

Though the term "tetraspore" is strictly applicable in this case, as in most of the Florideæ, still in *Callithamnion* the mother-cells may undergo a variable number of divisions, or may even remain undivided.

In many forms, with simple structure of the thallus, the tetraspores may be produced externally, as in *Callithamnion*, *Ptilota*, &c.

VIII. As above noted, the male plants may be recognized by the presence of yellowish tassels of antheridia, which are to be found mature from the beginning of April onwards: late in the season the ends of their branches become ragged and irregular. Mount as before a small piece of a male plant taken in April, and observe under a medium power—

1. The normal structure of the main thallus.

2. The club-shaped **antheridial branches,** often associated together in groups: each consists of—

a. A unicellular **pedicel,** by which it is attached to the thallus.

b. A central linear series of cells, which is almost entirely hidden by—

c. Numerous, closely aggregated, and small **antheridial cells.**

Mount specimens of antheridial branches from fresh living material in sea-water, and having found an antheridial branch exactly at the period of maturity observe the partial disorganization of the walls of the antheridial cells, and consequent liberation of their protoplasmic contents, without subdivision, as round non-motile **spermatia.**

A comparison of numerous antheridial branches in various stages of development will demonstrate that they originate from single cells of the thallus, close to the apex of the branch: these cells divide repeatedly by transverse walls, so as to form a linear series, around which, in place of the normal pericentral cells, antheridial cells are cut off by repeated longitudinal or oblique divisions from all the cells of the linear series, except the basal one, which remains as the pedicel.

Transverse sections should also be cut through the antheridial patches of *Lemanea,* when the antheridial cells may be recognized as oval buds borne on club-shaped pedicels.

IX. Having recognized a female plant by observations with a lens on specimens taken in late summer or autumn, mount a portion of it in glycerine, and, examining it under a low power, observe—

1. The normal structure of the thallus, which bears—

2. **Cystocarps** of ovate form: these consist of closely aggregated, small-celled tissue: they occupy the same position as the lateral branches in a vegetative thallus.

3. Compare numerous specimens, and note cystocarps in various stages of development.

By careful observation on material taken in early summer the various steps in the development may be traced, such as the origin of the cystocarp as a multicellular body, seated at a point immediately below the apex of a short lateral branch of the thallus, and recognized as a darker group of cells: the mode of segmentation of the young cystocarp, and the formation of the **trichogyne** as an elongated thread-like cell, which overtops the apex of the branch on which it is borne: its fertilization by a spermatium, and the further growth of the cystocarp.

As examples of simpler structure of the fruit may be suggested *Batrachospermum*, and *Polyides* or *Dudresnaya*, the latter genera being specially remarkable for the indirect character of the process of fertilization.

The case of *Lemanea* is worthy of note: the carpogonia are formed internally, but with a projecting trichogyne: in spring (March) these may be seen projecting from the surface of the thallus, and are perhaps as easily recognized as in any of the Florideæ. In summer the carpospores are to be found borne on branching filaments which occupy the large mucilage cavities in the thallus. Transverse sections of the thallus should be made so as to demonstrate the internal position, structure, and mode of development of the fruit, in material taken in early summer.

In the forms with more complicated structure, the cystocarp is often embedded in the tissue of the thallus: sections should be cut to illustrate this in *Chondrus crispus*.

X. Embed mature cystocarps of *P. fastigiata* in paraffin, and cut from them median longitudinal sections: mount in glycerine, and observe—

1. The short thick stalk of the cystocarp.

2. Its wall, consisting of small, closely aggregated cells, and with an opening or **ostiole** at the apex.

3. The central cavity, surrounded by the wall, and filled more or less completely, according to age, with elongated, club-shaped cells, having dark protoplasm, and swollen walls: these are the **carpospores.**

The following points are further to be observed in longitudinal sections, viz.—

1. The formation of the carpospores by budding from the base of the cavity of the cystocarp.

2. The contents of the spores in various stages of escape from their cell-walls, and of passage through the ostiole, so as to pass out freely, as rounded and naked protoplasmic bodies, from the cystocarp.

The artificial germination of the spores is a matter of difficulty, but a rough idea of the germination of the spores of red seaweeds may be gained by observing the numerous young plants, of various genera and species, which are to be found attached to the outer surface of almost any one of the larger seaweeds.

PHÆOPHYCEÆ

FUCUS SERRATUS, *L.* (Wrack)

OBSERVATIONS WITH THE NAKED EYE

I. Of the various species of *Fucus* which are to be found on our shores, the best adapted for laboratory work is *Fucus serratus :* it is to be found near or below mid-tide level, and may be distinguished from other species by its dark olive colour, the flattened form and serrate margin of the branches of the thallus, the absence of swollen "bladders," and the presence of numerous dot-like conceptacles, crowded together on the ends of branches which show no special swelling.

Having recognized the species by these characters, examine a well-developed plant with the naked eye, and note that the **thallus** as a whole shows no differentiation of stem, leaf, and root, as in vascular plants ; it consists of the following parts—

1. The flattened **disk,** of irregular outline, by means of which the plant is firmly attached to the substratum : the attachment is at times so firm that the stalk itself will break before the attachment gives way.

2. The **stalk,** which in old plants is of compressed cylindrical form, but in young plants it may be clearly seen that it is originally a broad flattened expansion, with a more or less thickened midrib: a comparison

of plants of successive ages will demonstrate that the compressed cylindrical stalk results from the thickening of the midrib, and decomposition of the lateral wings.

3. If this stalk be traced upwards it will be seen to branch repeatedly, while on tracing the branches also upwards they gradually assume the flattened form with serrate margin, thus confirming the conclusions which may be drawn from a comparison of younger plants, viz. that the whole thallus, when young, was of a flattened form, and that after growth and repeated branching the lower portions assumed the flattened cylindrical character, by thickening of the midrib and loss of the lateral wings.

The following observations are also to be made—

A. Examine the **apices** of young, actively-growing branches: those branches which bear conceptacles must be carefully avoided, as they do not show such characteristic appearances: the extreme apex is emarginate, or depressed, the base of the depression being somewhat flattened, and marked by a slight groove running in the plane of the thallus: it will be seen subsequently that the **initial cells** lie at the bottom of this groove.

B. Compare a number of apices: in some only a single emargination will be seen, in others two, similar to one another, each having the groove at the base, while others again will show an intermediate appearance: from this it may be concluded that the single apical point divides into two of equal strength, each of which may develop into a branch of the thallus similar to the original: thus the **branching is a dichotomy.**

C. On comparison of a number of branches it may be seen that the development of the two branches of a dichotomy is not equal, one being usually stronger than the other: this leads to a **sympodial development of the dichotomous branch-system.**

D. Observe the less regular outline of the ends of those branches which bear **conceptacles,** the latter being seen in large numbers on those branches: note with a lens the round orifice or **ostiole** in the centre of each conceptacle. When mature, two kinds of conceptacle may be recognized in this species: they are borne on different plants, and this species may accordingly be recognized as dicœcious: they are—

a. Conceptacles the contents of which are of a dark olive-green: these contain the **oogonia,** and are the **female conceptacles.**

b. Others with yellow or orange contents: these are the **male conceptacles,** and contain the **antheridia.**

Of other common species of *Fucus*, it is to be noted that in *F. vesiculosus* there are numerous air-bladders in the tissue of the thallus, that the conceptacle-bearing branches are swollen, and that the distribution of the sexual organs is as in *F. serratus*, the plant being dicœcious: in *F. platycarpus*, on the other hand, male and female organs are found on the same plant and in the same conceptacle, while the fertile branches are, as in *F. vesiculosus*, considerably swollen.

E. If those flattened parts of the thallus be examined which do not bear sexual conceptacles, there will be found, scattered here and there, organs of somewhat similar structure, which contain only barren hairs, and they may be termed **sterile** or **neutral conceptacles.**

F. Note the dark olive-brown colour of the whole thallus when fresh. Place a piece of the fresh thallus in methylated spirit: in a short time it will assume the bright green colour characteristic of chlorophyll: this is due to the fact that the brown colouring-matter (phycophæin, or phycoxanthin) characteristic of the Phæophyceæ, masks the chlorophyll in the natural state; but, being more readily soluble than chlorophyll, it is first removed, and the chlorophyll is then apparent.

MICROSCOPIC EXAMINATION

II. As it is almost impossible to make satisfactory preparations of the tissues of *Fucus* from fresh material, it will be found a great advantage to fix and harden them, by some such method as that described in small type on page 6: by this method the material is fixed by treatment with a solution of picric acid in sea-water, and after washing, to remove excess of picric acid, it is hardened in successive strengths of alcohol. The length of time of immersion in the picric acid should be proportional to the bulk of the tissue, so that the reagent may penetrate throughout the tissue.

An alternative method, which gives good results, is to treat the specimens first with 1 per cent. chromic acid, wash with water, and harden in successive strengths of methylated spirit.

From material thus prepared select a young flattened branch of the thallus in which the midrib is but slightly marked: cut transverse sections from it, mount in pure glycerine, and observe under a low power—

1. The elongated elliptical outline of the section.
2. The more or less enlarged midrib.
3. The grouping of the tissues exposed: recognize especially—

a. A compact marginal band of tissue of a yellowish-brown colour, the **cortical band:** this graduates off into—

b. The less compact central mass of the **medulla,** consisting of a web of interlacing filaments.

Put on a high power, and examine the tissues in detail. Starting from the periphery, observe that the cortical band consists of—

1. A superficial or **limiting layer** of cells, regularly arranged, and elongated radially; the cells are not of uniform depth, and examination will show that they divide by periclinal, as well as by anticlinal walls; in fact they constitute an active, continuously meristematic layer, and accordingly the term "epidermis" cannot be applied to it in the strict sense. With the exception of the outer wall, which is thick and cuticularized, the walls of these cells are thin, and the protoplasm plentiful, with a nucleus.

Treat a section with strong sulphuric acid: all the cell-walls swell, and lose their definite outline, with the exception of the outer layer of the superficial wall, which remains sharply marked. Treat a fine section with Schulze's solution: the cuticle will stain yellow, while the walls of the internal tissues stain a pale blue.

2. Immediately below the limiting layer, and separated from it by an irregular line, is a parenchymatous tissue, consisting of cells which appear nearly square in the

transverse section; each cell has plentiful protoplasm a nucleus, and several chlorophyll corpuscles: the walls are more or less thickened, swollen, and stratified: here and there are to be seen **pits** closed by a thin, highly-refractive pit-membrane. These two tissues, (1) and (2), together constitute the **cortical band** above recognized under a low power.

Here, as in other cases of pitted tissues, the question of perforation of the pit-membrane has been raised: though the actual proof is in this case difficult, it is asserted by various authors that the continuity of protoplasm through the pit-membrane has been actually observed.

3. The above tissue graduates off without definite limit into the **medulla,** of which the chief characteristic is the excessive bulk of the swollen cell-walls: an idea of the manner in which this comes about may be formed by following the gradual transition from cortex to medulla, when it will be apparent that the parts of the wall adjoining the middle lamella (and sometimes the middle lamella itself) swell greatly to form a gelatinous matrix, in which the cells appear to be embedded, each being surrounded by a definite, firm, cellulose wall. Here and there "trabecular" filaments will be found running in the plane of section. If sections be stained with Schulze's solution the firmer cell-wall stains pale blue, but the swollen matrix does not stain.

Occasionally a section may be found which has passed through one or more of the sterile or neutral conceptacles; these will then appear (if cut through the ostiole) as an irregularity of the cortical band. If the conceptacle was young, it would be found to be a still

closed cavity of considerable size, filled with mucilage, and with hairs, which originate from single cells of the tissue lining the cavity; in sections of older parts the ostiole would be found widely open, the hairs much longer, and protruding out of the conceptacle: these hairs may be observed even with the naked eye on the older parts of the thallus.

III. Cut longitudinal sections of a young part of a thallus (*i.e.* close to the apex of a branch), mount in pure glycerine, and examine under a high power: recognize, as in the transverse sections, the **cortical band,** consisting of—

1. The **limiting layer,** which presents similar characters to those seen in transverse sections.

2. The **inner parenchyma:** note that the cells of the inner part of this tissue are arranged in longitudinal rows, having relatively thick, occasionally pitted, lateral walls, and thin transverse septa: this tissue merges gradually into—

3. The **medulla,** which consists of **"trabecular" tissue:** here longitudinal rows of cells constitute filaments resembling the hyphæ of Fungi, with numerous thin transverse septa, to which the protoplasmic contents closely adhere: as already seen in transverse section, the longitudinal walls consist of a firm, highly refractive cellulose coat, embedded in a mucilaginous matrix. Note the frequent lateral communications of the filaments, and compare them with the pits in the cortical tissue, to which they undoubtedly correspond.

IV. In order to study the process of thickening of the midrib, cut transverse sections successively of older (*i.e.* lower) parts of the stalk: treat as before, and

compare them. It will then be recognized that, as the thallus grows older, the cells of the limiting layer cease to divide by periclinal, and later also by anticlinal walls: it becomes a quiescent tissue, and is ultimately thrown off; the inner cortical tissue however remains active, the cells increase in size, dividing periclinally, and form a massive band, easily recognized by the naked eye. The medulla also increases greatly in bulk, many new hyphal filaments being formed, while they differentiate into two series: (*a*) smaller ones, with sparing protoplasm; (*b*) others of larger size, with a granular protoplasmic lining.

Longitudinal sections made at different points down to the thickened stalk itself, will lend solidity to these observations: in such sections note, especially in the oldest parts of the stem, the different types of hyphæ; also the origin of fresh hyphal filaments from cells of the cortical band, as tubes which push their way through the mucilaginous cell-walls of the earlier-formed tissue. Look for endings of some of the filaments: note also the circular pits in their lateral walls.

V. Sections should be cut through the organ of attachment. Take plants grown on wooden piles, or on limestone rock: in the latter case the lime may be dissolved by acetic acid, and the tissue then hardened in alcohol. Cut vertical sections, and mount as before: note under a low power the irregularity of the surface of attachment, which closely follows that of the substratum, hence the firmness of its hold. Foreign bodies may often be seen embedded in this part of the thallus, and this finds its explanation in the fact that the tissue here consists of hyphæ similar to those of the medulla,

and each appears to grow in an independent manner. Examine the section under a high power, and it will be seen that the mass of tissue resolves itself at the surface of attachment into a number of separate filaments, each of which applies itself separately to the surface of the substratum. Compare drawings or preparations of young plants (see below, p. 405).

VI. Cut thin longitudinal sections through the apex in a plane perpendicular to that of the flattened thallus: treat those sections which are **median** with glacial acetic acid, and mount in a mixture in equal parts of pure glycerine and glacial acetic acid: examine under a medium power, and observe—

1. The outline of the section, showing a depression of the apex corresponding to the groove already recognized with the naked eye.

2. That from their arrangement it may be concluded that the various tissues of the thallus are derived from an initial point at the base of the depression.

3. Trace the cuticle as continuous from the free outer surface into the slightly enlarged cavity of the depression, which is itself filled with a mucilaginous secretion lying between the cuticle and the cellulose walls of the adjacent cells.

4. Examining the tissue at the base of the depression, if the section be median, a single large cell having the form of a truncated pyramid may be recognized in a central position: **this is one of the initial cells.**

Put on a high power, and observe the **form** of the initial cell and its relation to the adjoining tissues: it will then be noted that segments are cut off alternately from either side, and then

one from the base. The lateral segments undergo further divisions according to the type of the limiting layer; the basal segments divide up, and ultimately pass over into medullary tissue.

If sections through the apex be stained with hæmatoxylin, it may be observed that each cell contains a single **nucleus**: this observation may also be extended to the tissues of the older parts of the thallus.

VII. Cut successive transverse sections of the apex, so as to pass immediately below the base of the depression: one of these sections will include **the initial group,** which will then appear to be composed of some four or five cells, of oblong form, placed in a row, side by side.

Examining these under a high power it may be seen that segments are cut off (1) from either side of their oblong outline, (2) from the sides of those of the initial cells which are at either end of the row, and (3) that any of the initial cells may divide by a median wall into two similar cells: by the latter process the number of initial cells may be increased, and this is usually the case before dichotomy: this is brought about by the cells at the middle of the row ceasing to act as initial cells, and passing over into the condition of permanent tissue, while those at either end of the row retain the character of initial cells, and are to be recognized as the two independent initial groups of the new branches: thus the branching is a dichotomy. The attempt should be made to find sections illustrating this process of branching.

This type of apex is by no means constant for the Fucoids; thus in *Himanthalia* there has been found in each branch of the thallus only a single apical cell, which appears triangular in transverse section, but in longitudinal section it shows two convex sides: the form of the cell is thus, roughly speaking, that of a Brazil nut.

VIII. Cut transverse sections through the fertile branches of the thallus, so as to traverse the mature

conceptacles: mount in glycerine, and examine under a low power. First take sections of the male thallus, and having found a point where a male conceptacle has been cut in median section (*i.e.* so as to traverse the ostiole), note—

1. The spherical, or flask-shaped **cavity.**

2. The **ostiole,** by which the cavity communicates with the exterior.

3. The **hairs,** which almost fill the cavity, and may even protrude through the ostiole.

4. The **antheridia,** which are single oval cells, borne often in large numbers on these hairs.

It may be further noted that the cavity is lined by a small-celled tissue, from which these hairs arise, and that this graduates imperceptibly into the other tissues of the thallus, which are similar to those of the purely vegetative parts.

IX. Tease out with needles the contents of a male conceptacle in glycerine, mount, and observe under a high power: note—

1. The thin, colourless, branched hairs, which bear the oval cells (**antheridia**) with their yellowish granular contents.

2. The mode of branching of the hairs which bear the antheridia.

3. Long hairs, branching less frequently, or not at all; these do not bear antheridia.

X. With the above, compare sections cut through female conceptacles, mounting as before: in its form, and also in its relation to the tissues surrounding it the female conceptacle is similar to the male; the difference is in the contents, which may be seen to consist of—

1. Barren hairs, which are usually unbranched.

2. **Oogonia,** bodies of relatively large size, and oval form, with a thick transparent wall, and dark granular protoplasm: each of these is seated on a unicellular pedicel, and may be regarded as a metamorphosed hair.

Observe in the largest of the oogonia that the protoplasmic body may be seen to have undergone division into eight parts, the surfaces of separation being visible as transparent lines.

In order to follow the development of the conceptacle it is necessary to cut sections from the apical region of a branch in which the formation of conceptacles has recently begun: median longitudinal sections will be found to be the best, since they will often show a series of successively younger conceptacles leading up to the organic apex. From a comparison of these it will appear that the conceptacle is not a mere involution of the surface, but that one (or in some cases more than one) cell of the limiting layer shrivels, and is thrown off: that the walls around it undergo mucilaginous swelling, which is probably connected with the formation of the flask-shaped cavity: that the conceptacle is closed until after the hairs begin to be formed from the tissue lining it, when ultimately the ostiole opens to allow the escape of the sexual cells.

XI. Observations on the extrusion of the spermatozoids and ova, and on the process of fertilization in *Fucus* must be made with fresh material, and will be most successfully carried out on the coast, the best season for it being winter or spring. Those who have not opportunity for this may succeed in making the observations on fresh material sent from the coast, using a solution of Tidman's sea-salt, 5 ounces to the gallon, in place of fresh sea-water.

If specimens of *Fucus serratus* be kept exposed to

the air for some hours (the period of one tide will suffice), an exudation may be observed from the ostioles of some of the conceptacles: on male plants it will be of an orange colour, on female plants of a dark olive-green.

Taking first the male, mount a small quantity of the orange exudation in a drop of fresh sea-water, and examine it under a high power: it will be found to consist of numerous **antheridial cells,** separated from the hairs which bore them: they will be seen to be bursting, and setting free their contents, and the following stages of the process are to be noted—

1. The antheridial cell is completely closed, the contents are already divided into numerous elongated bodies (said to be sixty-four in number), each having one or sometimes two brightly orange-coloured globules (**chromatophores**): these are the **spermatozoids,** and they may be seen to be in motion before the antheridium bursts.

2. The wall of the antheridium consists of two layers, the outer more firm layer (**extine**) and the inner mucilaginous layer (**intine**): observe the extine to burst at one end, usually at the apex, and the contents inclosed in the intine escape from it.

3. The intine gradually swells, loses its contour, and the spermatozoids separate, as actively motile bodies of elongated pear-like form. Observe their movements.

To a drop of water containing motile spermatozoids add a little iodine solution, put on a cover-slip, and examine under a high power: the two **cilia** may be observed on each spermatozoid attached laterally.

the formation of this wall is the first obvious change after fertilization.

2. The elongated club-shape of the older plants.

3. Their terminal depression, from which numerous hairs protrude.

4. The mode of attachment by means of hypha-like threads of independent growth, similar to those seen in the older plants: some of these threads may be seen quite young, and not yet attached to the substratum.

From such plants as a starting-point, intermediate forms will lead on to the mature *Fucus* plant.

Laminaria digitata, *Lamour.*

The genus *Laminaria* is so prominent among the Phæophyceæ that it calls for a short notice: the various native species of the genus grow near to or below the low-tide mark, and *L. digitata* is perhaps the commonest of them all.

Note with the naked eye in fully grown specimens of this plant—

1. The **organ of attachment** at the base, which produces many branches: these approach the substratum, and attach themselves closely to it.

2. The elongated **stipe,** of more or less flattened cylindrical form, with a brown exterior and cartilaginous consistence.

3. The expanded **frond,** which is also cartilaginous, and is irregularly cleft into segments.

4. On some specimens, especially in winter, may be found irregularly thickened, slightly brownish patches, on which the reproductive organs are produced.

Cut through the stipe transversely, and, smoothing the surface, examine it with the naked eye: if the plant be an old one, there will be seen—

1. A brown peripheral band of outer cortical tissue.

2. A massive zone which constitutes the chief bulk of the stipe: in the centre of it may be recognized—

3. A medullary patch of narrow-oval form, which indicates the originally flattened character of the stipe itself.

From material previously hardened in alcohol, cut transverse sections of the stipe, so as to include all the tissues to the centre: mount in glycerine, and examine under a low power: note—

1. The superficial tissues of the **external cortex**, which may be more or less brown according to age, both in the walls and the cell-contents. In old specimens there will be found large **mucilage-cavities** at the inner limit of this tissue, which are apparently of schizogenetic origin.

2. A meristematic zone, or **cambium**, in which the cells correspond in appearance, and in their arrangement in radial rows, to those of the cambium of vascular plants: by the activity of this layer the stipe undergoes a **secondary thickening.**

3. Passing inwards, a bulky mass of tissue is found consisting of cells arranged with considerable regularity in radial rows, pointing to an origin from the cambium: the cell-contents are for the most part inconspicuous, and the walls of somewhat irregular thickness.

4. Centrally is the oval area or **medullary tissue**, above noted with the naked eye: the tissue here is traversed by numerous **hyphal threads**, similar in nature to those in *Fucus*.

Cut longitudinal sections of the stipe, and, treating as before, observe—

1. That the external cortex, cambium, and ground tissue consist of cells which are of no great length, with rounded or square ends, and walls with a few pits.

2. That the mucilage-cavities do not extend far in a longitudinal direction.

3. That the medullary tissue consists chiefly of hyphal threads closely interwoven, with thick lateral walls: they are divided by thin transverse septa, and are enlarged at the points where these occur (trumpet-hyphæ). These septa are perforated, and the proof of this may be attempted by use of the methods recommended elsewhere (pp. 115, 214). Note especially at the periphery of this tissue how the hyphal threads originate as papillæ from the cells of the

ground tissue, and then push their way as they grow between the neighbouring cells.

Cut transverse and longitudinal sections of one of the segments of the frond, being careful to avoid the reproductive patches. Comparing their structure with that of sections of the stipe, observe that the arrangement and character of the tissues is in the main similar: observe further—

1. That there is no definite epidermis (compare *Fucus*).

2. That the character of the cell-walls is like that in *Fucus*, the middle lamella being swollen, and the pit-membranes highly refractive.

3. Where the section has traversed the margin of one of the thong-like segments of the frond indications may be seen of their separation from one another by rupture, since the tissues are irregular at these points, and there is no superficial band such as is found over the rest of the surface of the thallus.

Cut sections through the reproductive patches, and mount in strong glycerine.

Here the superficial cells are more elongated than in the vegetative portion, thus forming a deep layer, in which two different constituents are to be recognized—

a. Narrow club-shaped cells, with swollen walls: these are the **paraphyses.**

b. Shorter cells with thinner walls, and more oval form: these are the "**sporangia,**" the granular contents of which may be seen to divide into a number of reproductive bodies, the further development of which is unknown.

CHARACEÆ

Various members of this family are found growing in stagnant, or slowly flowing fresh water: they are green, but owing to superficial lime incrustations, they may appear white and chalky, especially when dry: they are brittle in texture, and are commonly called Stone-Worts. They grow rooted in mud, and put up into the water a branched shoot, which may be a foot or more in length: this bears at intervals whorls of lateral appendages—the "leaves." In summer and autumn these bear the sexual organs in large numbers, the antheridia being specially prominent owing to their bright red colour. The odour of these plants is characteristic, being like that of onions.

There are two chief genera of the family, viz., *Chara* and *Nitella* (besides other subgenera): the most obvious difference between these is that *Chara* is more robust, the stem and leaves having a peripheral cortex, while *Nitella* has none. A large number of species are distinguished, and as it might be difficult for beginners to recognize any one definite species with certainty from others, the description given below will be confined to the more essential characters of the genus *Chara*, while *Nitella* will be dealt with incidentally in small type.

Fresh material should be used if possible; most of the structural points can, however, be successfully observed on material preserved in alcohol.

I. Examine a mature specimen of *Chara* with the naked eye, or with a pocket lens, and note—

1. The **stem,** which is as thick as coarse packthread, and is marked off into **internodes** of length varying from 1 to 3 or 4 inches: this axis is of unlimited growth, and is terminated by an apical bud.

2. The **"leaves"** or branches of limited growth, which are arranged in whorls, the point of insertion of each whorl being recognized as a **node:** the number in each whorl may vary. Examine the leaves with a lens, and observe that they also show a distinction of nodes and internodes; in some species whorls of small unicellular outgrowths (leaflets) may be seen at the nodes: thus the leaves repeat the characters of the axis on a smaller scale.

3. In the axil of one leaf in each whorl is usually found a **branch of unlimited growth,** which repeats all the characters of the main axis.

4. Examine the base of the plant where it is fixed in the mud: very long transparent rhizoids may be seen to be inserted at the nodes.

5. On the inner side of the leaves of plants taken in summer or autumn the **sexual organs** will be found, viz., the **antheridia,** which are globular and of a red colour, and the **oogonia,** which are of a dark olive colour or brown: these sexual organs are seated, in the monœcious species, in pairs, at the nodes of the leaves, and on the inner, adaxial, side of them.

The general features of *Nitella* are similar to the above: note however its more transparent appearance, and the presence of only one node on each leaf, while two axillary buds commonly appear in each whorl. They are sometimes incrusted with lime, but are commonly free from it. The sexual organs are borne in groups of three, including two antheridia and one oogonium.

II. Mount a young part of a plant, including at least one whole internode and two nodes, in water: examine under a low power, and observe:—

1. The cylindrical **stem;** the internode is covered externally by a small-celled **cortex,** which surrounds one very large **internodal cell.** Note that the cortex is composed of (*a*) elongated cells, and (*b*) short cells which project as hemispherical bosses, the whole being disposed in spirally curved rows.

The fact that the cortex invests a large central cell should be verified by cutting transverse sections: these may be prepared from fresh material, the internode being held in pith or carrot; but better results will be obtained by treating first with acetic acid to dissolve the lime which is often present in large quantities: then after hardening in alcohol the stem may be embedded in paraffin, and transverse sections be cut. The sections will show a large circular central cell, surrounded by a single layer of smaller cells of the cortex.

2. The **leaves,** which are also covered in their basal part with cortex, but the cells are straight or only slightly curved. Note that the nodes of the leaves may be seen to be marked by cells which project as round bosses (**leaflets**), or in some species they are elongated. The single cells of the cortex of the leaf extend half way between two successive nodes and at the middle of the internode, the cells meet end to end, the ends of those descending from the upper node alternating with those

which ascend from the lower. The cortex stops short below the apex of the leaf, which is accordingly terminated by a series of naked cells.

3. Follow the leaves down to their base of insertion at the **node,** and observe a series of short cells which project more or less below the point of insertion : these are the so-called "stipules." The axillary bud will also be seen inserted in the axil of the oldest leaf of the whorl.

A comparison should be made of the structure of some species of *Nitella,* and its simpler construction noted, involving no cortex either on stem or leaves. The single large internodal cell should be examined, and it will be seen to present a smooth wall, enclosing a colourless protoplasm, in the peripheral part of which are numerous green chlorophyll grains : these are so disposed that a spiral line may be seen running longitudinally, in which they are entirely absent : this is the neutral line as regards the protoplasmic movements.

III. Remove and examine a whole bud under a low power : either an apical or an axillary bud will do. It may be necessary to treat with acetic acid to remove the lime, which is often present in considerable quantity : the bud may be subsequently cleared with potash, and mounted in glycerine. The outer and older leaves will show the characters above noted, but more clearly, since they are younger, and their internodes shorter : the structure of the cortex will thus be better understood in the young than in the mature leaves.

Examine also the cortex covering the short, young internodes of the stem, and recognize the regular arrangement of the cells : the cortex of each internode is composed of two series of lobes, the one ascending from the lower node, the other descending from the upper :

these are in contact at the middle of the internode, and elongate with it as it grows. Each lobe is composed of nodes (3-celled), and unicellular internodes, which alternate in a manner similar to that of the stem itself: compare this arrangement with that of the mature cortex.

Having thus examined the bud, remove the outermost whorls of leaves with needles: then add a little potash and cover the remaining central part of the bud with a cover slip; press gently with a needle upon the cover slip, watching the effect under a low power. The outermost remaining leaves will be pressed aside, and the **apex of the stem** will be exposed.

A more exact and satisfactory method is to cut longitudinal sections through the bud: this may be done roughly from fresh material, by holding the bud between the finger and thumb, and slicing it longitudinally with a razor: the sections thus cut are to be mounted in water; or the apical buds may be hardened in alcohol, and embedded in paraffin: sections are then to be cut longitudinally, and they may be mounted in glycerine, or they may be stained with Hæmatoxylin and mounted in canada balsam.

Observe the terminal dome-shaped, **apical cell,** from which segments are cut off by transverse walls. By comparison of the terminal series of cells from several apices it may be concluded that each segment cut off from the apical cell divides again transversely into two, of which the lower cell without further division develops directly into an internode, the upper divides to form the numerous cells of the node from which are derived the leaves, and the cortex.

Compare this result with the appearance of the leaves when young.

Note that each cell contains a single nucleus.

It has been ascertained that numerous nuclei are present in the mature internodal cells, and they are derived by fragmentation from the original nucleus: evidence of this may be obtained from stained preparations.

IV. The cells of the Characeæ are well known as good material for showing the movements of protoplasm in the living cell: observations of this are to be made on living specimens mounted in water. *Nitella* may be used, or the naked terminal cells of the leaves of *Chara*. Note that the chlorophyll granules, which lie in the outer band of protoplasm, are stationary: the colourless protoplasm below shows however movements by which the granules and clots are carried along, so that a **rotation** takes place round the large central vacuole. If the movements be sluggish they may be accelerated by gently warming the slide. Note especially the movements in opposite directions on either side of the neutral line, also the relative movements of the contents of adjoining cells.

V. In order to see the rhizoids, which fix the plant at its base, remove one of the lowest nodes of an old plant: wash it gently from mud, &c., and mount in water: numerous long, transparent threads will be seen to spring from the node: these are the rhizoids. Observe their smooth wall, and granular protoplasm with central vacuole, and the more or less obvious nucleus. Here and there they branch, the point of branching being marked by an oblique, and peculiarly curved cell-wall: from a swelling above the septum the branch-rhizoids spring.

There are various modes of vegetative propagation of the Characeæ, by means of peculiar forms of branching at the nodes:

as these are not of very constant occurrence, reference should be made with regard to them to the descriptions in text-books.

VI. The sexual organs are first to be examined in the mature state: mount a leaf, bearing the bright scarlet **antheridia**, in water, and examine under a low power. Note the **position** at the node and below the oogonium: the spherical **form**, and attachment by a very short stalk. Observe also the surface markings, which indicate that the whole spherical wall is made up of eight unicellular **shields**, of which the four upper are triangular, but the four lower, adjoining the stalk, are four-angled.

Press gently on the cover-slip: the antheridium will burst, and disclose numerous closely packed **antheridial filaments**; each of these is partitioned transversely into numerous disc-shaped cells, and contains at maturity a single spiral **spermatozoid**: the form of the latter can be clearly seen under a high power, and under favourable conditions their escape as free, spirally coiled bodies, with two cilia.

Attention should be paid to the mode of attachment of the filaments to the shields: in a mature antheridium which has been burst by very gentle pressure, observe that an elongated cell, the **manubrium**, rises from the centre of the inner surface: this is terminated by a **head-cell**, which supports six secondary heads, and to each of the latter are attached four of the antheridial filaments: their total number is therefore about 200.

The development of the antheridium, or "globule," may be readily followed in the young bud by careful teasing out of its parts, and treatment first with potash, and subsequently with

weak acetic acid and glycerine. In such preparations it may be ascertained that the antheridium arises in place of an adaxial leaflet; from the single cell a **pedicel** is first cut off, and the head then divides into octants: each octant undergoes periclinal division, the inner cell again dividing. The outermost cells form the shields, the middle series the manubria, while the innermost series of eight give rise by growth, budding and repeated divisions, to the capitula, secondary capitula, and antheridial filaments. By careful staining of the latter in various stages of their growth illustrations of the development of the spermatozoids may be obtained.

The antheridia of **Nitella** are similar in structure to those of **Chara,** but differ in their position, being developed from the terminal cell of the leaf; accordingly it is easier to trace the development in **Nitella.**

VII. Mount in water a leaf bearing one or more mature but unfertilized **oogonia** or "nucules" as they are sometimes called, and examine under a low power. Observe their **position,** directly above an antheridium: their oval **form,** and insertion by a short pedicel. Each consists of an outer coat, composed of five spirally twisted cells, a crown or apical rosette of five cells, and a large central **ovum:** at the base of the latter when young, or after treatment with potash, a short cell is to be seen. Note that a narrow lateral slit may be seen between the cells of the crown at the receptive period, through which the spermatozoids may pass to the ovum. Attempts may be made to see the entry of the spermatozoids by adding a drop of water containing motile spermatozoids to a preparation in water of a mature oogonium.

Sections should be cut longitudinally through the oogonium: this may be done by hardening in alcohol, and embedding in paraffin: in sections thus prepared the details of internal structure, which are only seen

with difficulty in the whole oogonium, owing to the density of its contents, may be satisfactorily seen.

The development of the oogonium should be studied in young buds, teased out with needles (or cut in longitudinal section), and treated with potash: in these it will be seen how the five cells, which are to form the coat and crown, gradually grow over the central cell: that they are straight at first and become subsequently coiled, and that the terminal part of each is cut off to form the crown.

The essential characters of the oogonium of **Nitella** are similar, but the crown consists of *two* series of five cells, one above the other, and several small cells may be found cut off at the base of the ovum. The position of the oogonia is also different, two being usually disposed, right and left, below the terminal antheridium.

VIII. Examine mature oogonia and note the dark colour, and the thickened, lignified wall of the spiral cells, while the oospore itself is surrounded by a thick, colourless wall. Burst one by pressure upon the cover-slip, and it will be seen that the contents consist largely of starch and oil.

IX. The results of germination may be readily observed in *Chara* or *Nitella*, if specimens with mature oospores be kept in water in a bell-glass through the winter: in the spring the oogonia which had settled to the bottom may be found in various stages of germination. Some may be seen still closed at the apex: in others the spiral cells may have ruptured at the apex and two or more transparent filaments project: one of these develops more strongly as the **proembryo,** it divides by transverse walls, and assumes a green colour: the other remains colourless and develops into rhizoids. In one of the filaments which is far advanced observe a narrow, disc-shaped cell, two or three cells short of the

apex—this is the **stem-node**: from it a pseudo-whorl of leaves arises, and, as a lateral bud, the new *Chara* plant, which at once shows the characteristic alternation of nodes and internodes: observe its position, and successive stages of development.

Lower down is a second node—the **root-node,** which gives rise to rhizoids; these may branch and fix the young plant in the mud.

Further observations may be made on the details of segmentation of the germinating oospore, and the mode of origin of the lateral bud which gives rise to the new *Chara* plant: but for further information on such points reference must be made to text-books.

CONFERVOIDEÆ

COLEOCHÆTE SCUTATA, *Bréb*

Various species of *Coleochæte* are to be found growing closely attached to the surface of submerged fresh-water plants: they are green-coloured, and they attain such a size as to be recognizable with the naked eye. The thallus shows considerable variety of structure in different species: thus it may appear as a flattened disc, one layer of cells in thickness, closely attached to the surface of the plant on which it grows (*C. scutata*); or it may consist of a number of filaments branching in one plane only (*C. soluta*); or it may take a hemispherical massive form, being composed of closely packed, branching filaments (*C. pulvinata*). Of these species *C. scutata* is very common, and is best adapted for observation. Having ascertained that this plant is present (*e.g.* on the lower surface of the lamina, or on the petiole of the Water Lily) cut thin tangential sections, removing as little as possible of the tissue of the leaf, but taking care not to injure the Alga: mount in water, and examine under a low power. Note—

1. The flattened discoid thallus, with more or less irregular circular outline: it consists of one layer of

cells, with thin cell-walls, a single nucleus, and a single flattened chlorophyll-body (chromatophore).

2. The arrangement of the cells in radiating and bifurcating series, which are however in close contact laterally one with another, and thus compose a continuous disk.

3. The hairs, which are borne by many of the cells: they are long and narrow, and are sheathed at the base.

According to circumstances, the following reproductive organs may or may not be found:—

1. **Swarm-spores,** each of which is formed from the contents of a single cell of the thallus, by contraction, and escape through a hole in the cell-wall into the water: the swarm-spores are spherical primordial cells; they move for a time by means of a pair of cilia.

2. **Antheridia,** which are formed by division of cells of the disk into four: the contents of these escape as ciliated **spermatozoids.** In other species they may be formed from terminal cells of the filaments (*C. pulvinata*).

3. **Oogonia,** flask-shaped cells, with long tubular necks: each oogonium contains one **ovum.**

4. The **fructification,** which results partly from the maturation of the oogonium after fertilization (**oospore**), partly from its investment by filaments which grow from surrounding cells, forming a sheath one layer of cells in thickness, and of a brown colour when ripe. In this state the winter is passed.

5. In specimens observed in spring the general outline of the thallus will be seen as before, but the cells, excepting those of the fructification, have lost their

contents. Cell-division now takes place in the oospore to form numerous cells, from each of which a swarm-spore may be produced. These may escape by disorganization of the investments, and, after a period of movement, settle, form a cell-wall, and grow into a new thallus.

The observation of (1), (2), and (3) may be made in early summer; that of (4) in autumn or spring.

ŒDOGONIUM

Various species of plants belonging to this genus are to be found growing in fresh water: they are green, filamentous, unbranched Algæ, and are attached at the base to the external surface of submerged plants, stones, &c. The apex of the filament is in some species extended into a thin, hair-like process: there is often considerable irregularity in the thickness of the filament, by which character, as well as by the peculiar transversely striated markings of the cell-walls, these plants may be distinguished.

I. Mount filaments of *Œdogonium* in fresh water, having gently scraped them off from the surface to which they were attached, and examine them under a low power: observe—

1. The long unbranched filament, of uneven thickness, terminated at the apex either by a rounded cone, or by an attenuated process: note also at the base the **irregularly lobed disk of attachment.**

2. The **septa,** dividing the filament into a series of cells, with green-coloured contents.

3. At the upper ends of many of the cells are to be

seen **transverse striæ**: these are indications of past cell-divisions.

Examine the filaments under a high power, and pay special attention to these striæ and other irregularities of the otherwise smooth cell-walls. It will then be seen that the striæ are small, sharp-edged, ring-like projections on the outer surface of the wall: also that a single corresponding stria is to be found, more or less distinctly marked, at the base of each cell.

In some cells there may be seen an **annular ingrowth of the cell-wall** immediately below the striæ: note its form and connexion with the cell-wall; also, when seen in optical section, a central, dark mark: it is here that the ring splits, and by stretching of the ring the well-known process of **intercalation** of a new zone of cell-wall follows. Examine actively-growing filaments, and try to observe various stages of this peculiar process, noting also any indications of cell-division which follows it, the new septum being formed immediately below the thin-walled intercalated zone.

Passing to the examination of the cell-contents, observe—

1. The colourless **protoplasmic basis,** in which are embedded—

2. The **chromatophores,** which appear as elongated and branched rod-like bodies, more or less closely and irregularly connected together: here and there will be seen highly refractive **pyrenoids** attached to the chromatophores: these are clearly to be distinguished by their dusky purple staining on treatment with iodine solution.

3. A single **nucleus** in each cell, which is, however, difficult to recognize in well-nourished cells.

4. A large central **vacuole.**

II. An examination of fresh filaments may result in the observation of the reproductive organs, and numerous specimens should be looked over with the object of finding them. Thus the reproduction by **swarm-spores** may be seen, especially in the morning: without the cell having undergone any change of form the cell-wall ruptures by a transverse split, and the protoplasmic body, having previously contracted, escapes through the slit as a motile pear-shaped, primordial cell, the anterior end of which is surrounded by a fringe of cilia. After a motile period these attach themselves by the anterior end to some firm body, and, forming a cell-wall, develop into new filaments. Note young plants in early stages of germination: they may be found in numbers attached to submerged plants or stones in waters where *Œdogonium* grows.

III. There is some variety in the details of development of the sexual organs in different species of *Œdogonium*: some species are monœcious, others dicœcious.

The **oogonia,** or female organs, are most easily observed, being spherically enlarged cells of the filament, borne singly or several together: in such oogonia note—

i. The rupture of the cell-wall at the period of maturity by a transverse slit.

ii. The beak-like canal, which projects in some species from the slit.

iii. The hyaline receptive spot.

iv. In old oogonia the mature **oospore** with thick wall, and dense contents.

The **antheridia,** which are smaller and shorter cells than those of the normal filament: each divides into two cells, the contents of which, without further division, escape as a motile yellow **spermatozoid** similar in form to the swarm-spore. Attempts should also be made to observe the germination of the oospore.

With *Œdogonium* it will be well to compare species of *Bulbochæte,* which resembles *Œdogonium* in its fresh-water habit, its mode of attachment, and in its processes of cell-division and reproduction; but differs from it in its profuse branching, and in the presence of peculiar long bristles with swollen bases, which are borne on the ends of the branches.

ULOTHRIX ZONATA, *Ktz*

This Alga is to be found, especially in spring and early summer, in slow-flowing streams, and ponds of fresh water, or in cattle troughs, and fountains: it occurs attached to stones and other objects near to the surface, and is to be recognized as a delicate, filamentous, unbranched organism of a bright green colour: it is attached by an attenuated base to the substratum, and the simple filaments are composed of numerous cells, each having a median zone-like chromatophore; hence the specific name.

Mount some filaments of the Alga in fresh water, and cover with a glass slip: neglecting for the present those specimens in which the reproductive processes

are going forward, note in those which are growing in a vegetative manner the following structural points :—

1. The cylindrical unbranched form of the Alga.

2. The **septa,** dividing the filament into a number of short cylindrical, or slightly barrel-shaped cells, of more or less unequal length. Note cases where cell-division is in progress, or only recently completed.

3. The **base of attachment,** which is usually attenuated, colourless, and occasionally branched so as to obtain a hold on the substratum : granules of soil, &c., will usually be found attached to it.

4. The smooth **cell-walls,** consisting of a thin, outer, mucilaginous layer, and an inner, denser layer immediately surrounding the protoplasm.

5. The **protoplasmic body,** in which will be found—

a. A single **nucleus,** in a lateral position, and more easily recognized after staining with an iodine solution.

b. A median zone-like chlorophyll body, or **chromatophore,** which has the form of a flattened but divided ring.

c. A central vacuole.

Associated with *Ulothrix* will often be found the following Algæ which are allied to it : viz. *Stigeoclonium*, of which the filaments are similar to those of *Ulothrix*, but differ in being profusely branched ; and *Chætophora*, which also consists of branched filaments, but they are connected by their swollen cell-walls, into a more or less spherical gelatinous mass.

The reproductive processes are best seen in spring or early summer, and are encouraged by a gentle rise of temperature: they may be best observed in material

brought from outside in the morning into the higher temperature of the laboratory or working-room. There are two modes of reproduction of this Alga by means of **zoospores,** and they are readily distinguished by the size of the motile cells. Thus there are to be recognized—

1. The **macro-zoospores,** or asexual reproductive cells: these are relatively large, spherical or pear-shaped, green, primordial cells, furnished with four cilia attached to the hyaline anterior end, in which there is also a contractile vacuole. According to the bulk of the filament, one, two, four, or perhaps sometimes eight of these may be produced from a single cell: observe how they escape by a lateral hole in the cell-wall, being surrounded for a time by a protoplasmic membrane from which they soon free themselves, and begin active movement: finally they settle, form a cell-wall, and germinate directly to form new individuals.

2. The **micro-zoospores** or sexual gametes: these are relatively small; and according to the bulk of the filament, eight, sixteen, or thirty-two of them may be produced from each cell: observe how they also, after passing through a lateral hole in the cell-wall, are at first enveloped in a thin membrane, which is soon burst, and they escape as small pear-shaped motile bodies with two cilia. Observe carefully how these **conjugate** in pairs, coalescing first by their hyaline ends, then along their whole length; the resulting **zygospore** still continues to move by its four cilia for a time after conjugation, but finally settles down, and forms a cell-wall in about two days.

It will be difficult to observe the further growth

and development of the zygospore, since it undergoes a period of rest during the summer months, and its progress is very slow during the succeeding winter.

Preparations showing active zoospores should be treated with iodine solution, by which they will be killed, and the observation of the **cilia** will be easy by reason of the staining: note the four cilia in the macro-zoospores, while there are only two in those micro-zoospores which have not conjugated.

Trace the processes of change in the cells of the filament preceding the escape of the zoospores: according to Dodel and others their formation is by a process of cell-division: Strasburger, however, states that the process is simultaneous; being a case of free cell-formation, preceded, however, by a process of nuclear division.

CLADOPHORA

Various species of *Cladophora* are to be found inhabiting fresh or salt water: perhaps the commonest, and the most easily recognized, is *Cladophora rupestris*, which grows at all levels between the tide marks on rocky coasts. Its coarse and rigid filaments are of a deep green colour, and are profusely branched, so that the plants as a whole appear as dark green tufts, easily recognized after being once seen. The plant may be examined fresh, and be mounted in sea water; or, if necessary, material preserved in weak alcohol may be used.

Having mounted a small piece of the plant, examine it under a low power, and note—

1. The irregular cylindrical form of the filaments, which are divided by transverse septa into cells of considerable length: they are profusely branched, and two or three branches may be inserted at one point, the insertion being lateral and just below a septum.

2. The mode of origin of the branches may be seen to be by bulging out of the cell-wall laterally below a septum, the outgrowth being subsequently cut off by a transverse septum at its base.

Under a high power observe the following :—

1. The thick stratified cell-wall.
2. The protoplasmic body with a large central vacuole.
3. The chromatophores, of which the polygonal outline is difficult to distinguish : pyrenoids are present.
4. There are a number of nuclei in each cell, and they lie internally to the chromatophores, and project into the vacuole : they may best be seen in specimens bleached in alcohol and stained with methyl green or hæmatoxylin.

In summer many of the cells towards the ends of the filaments will be found empty, and with a round hole (ostiole) in the wall : these are empty zoosporangia. In others the protoplasmic body will often appear more dark and granular : such cells will show on examination various stages of production of the zoospores, which when mature escape through the ostiole formed by swelling and subsequent rupture of the cell-wall.

Zoospores of two sizes have been observed ; larger ventral zoospores which serve for vegetative propagation, and smaller *gametes* which conjugate. Observations should be made of these and their movements : the details of their structure may be made out by treatment with iodine.

SIPHONEÆ

VAUCHERIA SESSILIS, *Vauch*

I. This Alga is to be found growing as a lax green felt on the surface of moist soil (frequently on the soil in pots in greenhouses): it is of so coarse texture that the separate filaments can readily be recognized with the naked eye, having a somewhat dull-green, glassy appearance. Remove a small portion of this felt: tease it out as gently as possible in water, and examine it under a low power: observe—

1. The coarse, green, cylindrical tubes which constitute this Alga.

2. The absence of septa as a rule, though septa may be present occasionally in unhealthy specimens, and are formed in connexion with the reproductive processes.

3. The very irregular, and far from frequent, monopodial branching, and the rounded ends of the filaments.

4. Some branches may develop as **rhizoids,** ramifying in the soil, but these are frequently absent altogether.

5. There may be present lateral outgrowths of

peculiar form, which are the organs of sexual reproduction (**gametangia**), viz.—

a. Curved cylindrical bodies, which are the **antheridia.**

b. Obliquely oval, sessile bodies which are the **oogonia.**

In this species the sexual organs are associated together in groups of two or three, each being inserted separately upon the thallus: a single antheridium is usually associated with one or two oogonia.

It will be necessary to distinguish the species above named both from other organisms not nearly related to it, and also from other species of the same genus. Thus, the Moss protonema is usually found growing on the surface of pots, but it is readily distinguishable by its septa and mode of branching: other septate filamentous Algæ may also be associated with it, but are easily distinguished by their size, colour, septa, &c. Of other species of the genus *Vaucheria*, that most likely to be present is *V. terrestris*, which may be distinguished from *V. sessilis* by the antheridia and oogonia being inserted together on a common pedicel: other species differ in the insertion, number, and form of the antheridia and oogonia.

II. Put on a high power, and examine the structure of the thallus in detail. Note—

1. The smooth continuous external **cell-wall:** this may be made more apparent by plasmolysing some filaments with a 2 per cent. solution of common salt.

2. The **protoplasmic membrane,** which lines the wall, and incloses a large central vacuole which runs the whole length of the filament: this membrane may also be more readily distinguished in plasmolysed specimens. In the membrane are imbedded—

3. Numerous oval, or spindle-shaped **cholorphyll**

corpuscles: look for some of these undergoing division.

4. Round highly refractive **oil globules,** which are more or less numerous according to the condition of the plant as regards nutrition.

In addition to the above bodies embedded in the protoplasmic membrane, numerous small bodies, which have the characters of nuclei, are to be found; but in order to see them careful staining is necessary, and the following method is recommended for this purpose: treat fresh specimens with a deeply coloured solution of methyl-green in 1 per cent. acetic acid: wash quickly with 1 per cent. acetic acid, and mount in the same. Examination under a high power will show the presence of numerous small, stained bodies lying in the stratum of protoplasm immediately within that containing the chlorophyll corpuscles: on the ground of their staining reactions these are described as **nuclei**: the most satisfactory results are to be obtained in filaments in which the chlorophyll corpuscles are not densely crowded, or from the examination of the ends of growing filaments. The specimens may be permanently mounted in a mixture of weak glycerine and acetic acid.

III. On specimens which have been kept under conditions favourable for strong growth, the sexual organs (**gametangia**) are usually to be found in greater or less numbers. Having found a specimen with mature sexual organs, examine first the **antheridium** under a high power.

1. The lower straight portion, or pedicel, rises vertically from the main filament, its contents are in direct communication with those of the filament, but are separated from those of the antheridium proper by a transverse septum.

2. The curved portion, or **antheridium** proper, differs from the pedicel in the contents being for the

most part colourless: when mature the contained protoplasm forms a large number of small **spermatozoids,** which escape through an opening which appears at the apex.

By a comparison of a number of specimens various stages of development of the antheridium may be observed: *e.g.* (1) its origin as a rounded papilla from the main filament; (2) the appearance of the septum; (3) the opening of the pore at the apex, and escape of the spermatozoids; (4) the empty wall of the antheridium with the pore at the apex through which the spermatozoids have escaped; (5) the form of the spermatozoids and their cilia are to be observed in specimens stained with iodine solution.

Next examine a mature **oogonium** in detail: note its sessile position, and the septum which separates it from the main filament: its oblique form and green-coloured, granular contents: when actually mature an obliquely lateral beak is formed, the apex of which becomes gelatinous, so that the motile spermatozoids can gain access to the **ovum.**

By comparing specimens of different ages the following points may be observed: (1) the origin of the oogonium as a rounded outgrowth from the main filament; (2) the appearance of the septum; (3) the opening of the apex of the beak, and extrusion of a portion of the protoplasmic body, leaving exposed the colourless receptive spot; (4) attempts should be made to observe the actual entry of the spermatozoids.

It is stated that the development of the sexual organs takes place at night, and the process of fertilization during the day, the whole being completed within the twenty-four hours.

IV. Observe the changes which succeed fertilization, resulting in the formation of the ripe **oospore:** the chief are—

1. Formation of a firm wall completely surrounding

the fertilized **ovum,** and fitting closely within the wall of the oogonium.

2. An increase in the size and number of the oil globules: no cell-division takes place.

V. The reproduction by means of zoogonidia may readily be observed in specimens grown under favourable conditions in water: a considerable mass of the Alga is to be placed in a porcelain bowl, in water, and exposed in a window: after a few days, numerous small plants of *Vaucheria* will be found floating on the surface of the water, or disposed along the submerged surface of the bowl: these have resulted from vegetative reproduction by **zoogonidia.** In order to see the process observations must be made in the early morning, or else the culture must be kept in the dark till shortly before the observations are to be made. Shortly after dawn (or soon after the specimens have been exposed to light) some filaments may be seen with a lens to have dark-coloured and slightly swollen ends: these are about to form zoogonidia: mount some specimens without a cover-slip, taking care to avoid injuring them; and examine under a low power: observe—

1. The swollen end of the filament, with dark, densely aggregated protoplasm, surrounding a vacuole.

2. The transverse **septum** dividing the swollen end from the rest of the filament.

If such specimens be kept under observation the escape of the zoogonidium may be observed: the following points are to be specially noted—

1. Various changes in the protoplasmic body and vacuole, terminating in the formation of a transparent, and radially striated, outer protoplasmic coat (**ecto-**

plasm), which lines the cell-wall, while darker, more granular protoplasm (**endoplasm**), including the chlorophyll corpuscles, collects towards the centre.

2. The rupture of the cell-wall by an irregular slit near to the extreme apex of the filament: before the rupture the septum may be seen to present a convex surface to the rest of the filament, indicating greater internal tension in the "zoosporangium": on rupture this is relieved, and the septum then projects convexly into the cavity of the empty zoosporangium.

3. The passage of the protoplasmic mass through the opening, by a streaming movement, assisted by more or less marked, screw-like rotation of the whole body: the protoplasm may undergo division at the time of escape, and two zoogonidia may thus be formed.

4. The rapid movement of the large zoogonidium when free, which may be followed with the naked eye, and under the microscope is seen to be rotatory. The motile period lasts but a short time, and varies in different species.

Treat a zoogonidium, which has just escaped and is in rapid motion, with a solution of iodine: put on a cover-slip, and examine under a high power: no cell-wall will be visible, though the solution will in some measure plasmolyse the cell. Turning more especially to the ectoplasm, there will be seen numerous **cilia,** arranged in pairs, projecting from the surface of the zoogonidium, while in the transparent ectoplasm will be seen numerous highly refractive bodies, which stain with iodine: these are **nuclei,** and a careful observation will show that their position is exactly opposite the insertion of the pairs of cilia.

This method of demonstration of the nuclei has the advantage of simplicity, but the results are not permanent ; if it be desired to obtain specimens for keeping, they should be stained, as above directed, with methyl-green and 1 per cent. acetic acid ; or better with hæmatoxylin.

Treat a zoogonidium, which has come to rest, with a plasmolysing agent such as 2 per cent. solution of common salt, watching it meanwhile under a high power: the protoplasm will contract, and a fine **cell-wall** will be seen. This result may also be obtained by pressure on the cover-slip.

Mount and examine zoogonidia which have already come to rest, and by a comparison of them the process of germination of the zoogonidia, and the development of new plants from them may be deduced.

For comparison with *Vaucheria* another member of the Siphoneæ may be examined, viz. *Codium tomentosum*, which is to be found growing near low-water mark on rocky shores, and is an Alga of very wide distribution, though not of very general occurrence on the British coasts.

Observe externally the cylindrical, green, spongy thallus, which branches dichotomously, and is attached to the rocky substratum by an extended disk.

Transverse sections should be cut : if fresh material be used, they may be mounted in sea-water ; if alcohol material, in weak glycerine. Note, under a low power, the central felt of interwoven tubes, which are relatively narrow, and occasionally septate : the peripheral part of the section is occupied by a band of larger elongated sacs, arranged in a radiating manner.

Tease out a small piece of the thallus gently with needles : it will then be seen that the thallus consists of a system of branched tubular sacs, similar to those of *Vaucheria*, but aggregated together to form a **spurious tissue**, as is the case in the larger Fungi.

If the material be suitable, the sexual organs (**gametangia**)

are to be found in the form of short cysts, borne as lateral branches among the radiating peripheral sacs of the thallus, and completely overtopped by them. According to their size, smaller male (**antheridia**) and larger female (**oogonia**) are to be recognized on different individuals.

Observations may also be made on specimens of the native genus *Bryopsis*, which is to be met with occasionally on rocky shores.

If specimens be at hand, comparison should also be made of various species of *Caulerpa*, in which the thallus attains high complexity and varied conformation, though it is not partitioned into cells: in this genus reproductive organs have not yet been observed.

Acetabularia, which is a native of southern seas, and *Botrydium*, which is to be found growing on moist soil in swampy places, may also be examined with advantage if material can be obtained.

VOLVOCINEÆ

VOLVOX GLOBATOR

This Alga is to be found swimming freely in fresh-water ponds, mostly in exposed situations: it is of such a size that its simple spherical body can be recognized with the naked eye.

Mount one or more in water in a hollow-ground slide without a cover-slip, and examine under a low power: observe—

1. The hollow spherical form of the whole organism (**cœnobium**).

2. Its rotating movements in the water.

3. The tissue of the hollow sphere, consisting of numerous cells forming a single layer, and for the most part similar to one another.

4. The **daughter-cœnobia** of various sizes in different specimens: their number is usually **eight.** They may be seen projecting into, or even almost filling, the cavity of the parent.

Mount a cœnobium in water under a cover-slip, and examine it in detail under a high power: it will of course be squeezed flat by pressure of the cover-slip: in the tissue thus flattened observe—

1. The ordinary cells of the cœnobium, each consisting of a protoplasmic body with green **chromatophore,** a

red spot, and a slowly pulsating vacuole: each cell is furnished with two **cilia,** which project beyond the surface of the cœnobium: these are better seen after staining with iodine.

2. The fine threads of protoplasm, also seen more distinctly after staining, which traverse the swollen cell-walls, and connect the cells together as a continuous network.

3. At the extreme outer surface of the cœnobium note a more clearly defined membrane, marked off into polygonal areas corresponding to the cells: in this is the swollen gelatinous substance which fills the whole cavity of the cœnobium.

4. The cells which form the **daughter-cœnobia:** these may be recognized as of larger size than the rest, the protoplasm being denser. Observe in different specimens their various stages of division, first into four, then into eight cells: the disk-like group thus formed becoming convex (by reason of quicker surface-growth at the centre than at the periphery), and gradually assuming the form of a hollow sphere, which projects into the cavity of the parent: the **pore,** corresponding to the margin of the original disk, may be seen after the young cœnobium has assumed the spherical form, and even when it has attained a considerable size.

During the summer nothing further will be observed as to the mode of reproduction of *Volvox* beyond that vegetative propagation above described, which is repeated through a series of generations; but in the autumn **sexual organs** may be formed, the **antheridia** and **oogonia** being borne on the same individual in *V. globator*, and are easily distinguished from one another, and from the neutral cells of the cœnobium.

i. The **antheridia** are to be recognized as enlarged cells with

disk-shaped contents ; the disk breaks up into a large number of rod-like bodies arranged like a bundle of cigars. These may, under favourable circumstances, be seen to separate, and escape from the antheridium into the cavity of the cœnobium as spindle-shaped **spermatozoids** having two cilia attached laterally.

ii. The **oogonia** are easily recognized as enlarged flask-shaped cells which project into the cavity of the cœnobium.

iii. The result of fertilization is the **oospore** : these may be seen in considerable numbers in a single cœnobium : they are of spherical form, and show a thick cell-wall, developed inside that of the oogonium : the wall consists of an **epispore,** a firm membrane, with numerous superficial spines, and an **endospore,** which is a smooth layer. The germination has been observed in *V. minor* to result in the formation of a new cœnobium by a process similar to that in the vegetative reproduction : the same is presumably the case in *V. globator.*

If material be at hand, observations should also be made on *Pandorina,* the freely-swimming cœnobium of which is simpler in structure than that of *Volvox,* consisting only of sixteen cells. The sexual process consists in the conjugation of two freely-swimming, and similar swarm-cells.

PLEUROCOCCUS VULGARIS, *Meneg*

(*Protococcus viridis*)

This is the organism which is universally to be found forming a bright green pulverulent incrustation on the bark of trees, wooden rails, &c. If a small quantity of it be mounted in water, and examined under a low power, it will be seen to consist of cells with green-coloured contents ; occurring sometimes solitarily, but more frequently in groups of two, or four, or even larger numbers. Examination under a higher power will show that they multiply by division, and that

the resulting cells tend to round themselves off. Note especially that in groups of four or more cells, a splitting of the cell-wall at the centre of the group is frequently to be observed, which is doubtless the result of the tendency of the cells to assume a spherical form.

Select a single large cell for examination under the highest power, and observe the following details—

1. The **cell-wall** is thick, and shows clearly a double contour: staining with Schulze's solution gives a blue coloration of the cell-wall.

2. The protoplasmic contents, which at first sight appear uniformly green, will show differentiation, especially after staining with iodine solution: thus there may be recognized—

a. A **nucleus,** which is usually central, though sometimes it is in a lateral position.

b. **Chromatophores,** a number of which together form a partial envelope surrounding the nucleus: there are no pyrenoids.

c. A very scanty colourless **protoplasmic basis,** in which these bodies are embedded.

It is held that *Pleurococcus* is the resting stage of *Chlamydomonas*, an organism which differs from other Volvocineæ in that its motile cells are separate, and do not form a cœnobium: there is, however, still some uncertainty as to the details of the life-history of this organism. Material of *Pleurococcus* should be kept in water exposed to light, and observations made from time to time to see the motile cells, with their limiting cell-wall and active cilia: the latter are best seen after staining with iodine solution.

Hydrodictyon utriculatum, *Roth*

Observations may with advantage be made on *Hydrodictyon*, because of the very peculiar formation of the tissue composing its network. It is not, however, of very frequent occurrence: and it is to be found floating as a hollow sac-like net in still, fresh water in summer. Observation with the naked eye will show obviously the meshes of the net-work.

Mount a small piece in water and examine under a low power: the four- to six-angled meshes will be seen to be limited by as many cells, which are of large size. Each cell contains numerous nuclei, as may be ascertained by suitable staining. When a new network is to be formed, the protoplasm of one of these cells divides simultaneously into 7,000 to 20,000 parts; these move about within the original cell-wall, and finally arrange themselves in a new net-work, which, on growing, is liberated by rupture of the parent cell-wall. This process may be observed in healthy specimens in the height of summer.

The whole cycle of life is a complicated one, and is somewhat difficult of observation: as the organism is a rare one the various stages will not be described at length.

CONJUGATÆ

SPIROGYRA

1. In summer there may frequently be found, in stagnant or slowly-flowing waters, flocculent freely-floating masses of a vivid green colour, and slimy to the touch: with the naked eye it may be seen that the masses consist of coiled and tangled unbranched filaments, in which there appears to be no distinction of apex and base.

Mount a few of them in water, and examine them under a low power: note that the simple unbranched filaments are partitioned off by transverse septa into a number of relatively short cells. It will usually be obvious that the filaments are not all alike, and two chief types will frequently be found present—

a. Those with two star-like green bodies in each cell: these belong to the genus *Zygnema*, and, as these Algæ are not so well fitted for a detailed observation, they may be neglected.

b. Others will be seen to have one or more spirally coiled green bands in each cell: these belong to the genus *Spirogyra*.

A superficial observation of specimens collected at the same time and place will usually show that in different filaments there is considerable variety in size,

form of the cells, thickness of the walls, and in the number and arrangement of the spirals: according to these characters (together with those of the zygospore) a large number of species of *Spirogyra* are distinguished. It will be found convenient to select for observation specimens of the largest size, and with the coils of the green spirals furthest apart. Examine such filaments in detail under a high power, in the living state, mounted in water, and observe—

1. That the whole filament is covered externally by a transparent **gelatinous sheath,** with a somewhat irregular outer surface, and showing a radial striation: this is stated by Klebs to be an excretion from the protoplasm, not a result of metamorphosis of the outer layer of the cell-wall. It is to this layer that the Alga owes its slimy character. It is to be noted, however, that this sheath is almost entirely absent in some species.

2. A firm **cell-wall,** which is more highly refractive: it immediately surrounds the protoplasmic body, and is continuous with the transverse **septa.**

In respect of the nature of the septa there is some variety in different species, and the whole genus may accordingly be divided into two sections: (i.) those in which the septa appear as simple lamellæ, and the ends of the cells are then truncate; (ii.) those in which the septum is split in the central part of its area into two layers; these separate from one another and appear as two involutions, encroaching on the cavity of the cells. A sort of vegetative multiplication of these plants takes place by breaking up of the filaments, accompanied by splitting of the septa: in the species of section (ii.) the involute walls are then pressed outwards by the turgescence of the cells, and become convex: this may often be observed by moving the cover-slip while gently pressing on the object

3. The **protoplasmic body,** which consists of—

a. A colourless membrane (**primordial utricle**) which lines the cell-wall internally, and surrounds the large central **vacuole.**

b. The green spiral **chromatophores** (one or more) embedded in the protoplasm: note their irregular outline, and the numerous highly refractive lenticular bodies (**pyrenoids**) which are contained in them.

c. A bi-convex lens-shaped **nucleus,** suspended in the centre of the vacuole by fine colourless strands of protoplasm, which run to the primordial utricle, attaching themselves to points opposite the pyrenoids.

Stain with an iodine solution, and observe that the colourless protoplasm stains pale yellow, the nucleus a deeper yellow, and it will thus be more clearly seen, as well as one or sometimes two **nucleoli** which are deeply stained: the pyrenoids stain a dusky purple.

A careful comparison of these pyrenoids under high powers, with and without iodine staining, will lead to the conclusion that they are highly refractive, colourless bodies, around which is usually present a coating of starch, either as a continuous sheath, or in distinct granules. Such bodies are to be found in the chromatophores of many Algæ. Look for examples illustrating their multiplication by fission. In material decolorised in alcohol, or fixed by some other method, apply such staining reagents as hæmatoxylin and carmine: the pyrenoids will stain in a manner similar to nuclei. But the best method for bringing out clearly the structure of the pyrenoids is that of fixing and staining with picro-nigrosin (see Appendix A): after staining wash in alcohol and mount in weak glycerine: both the nuclei and the pyrenoids will be stained, while the starch-sheath remains colourless.

II. The process of cell-division may be very well observed in the filaments of *Spirogyra;* the chief

difficulty is however that the process normally takes place at night, beginning about 10 to 12 P.M. Strasburger, who has made this a subject of careful study, recommends that the *Spirogyra* be placed in a flat plate upon a block of ice during the night: if on the following morning the plants be exposed to a higher temperature, the cell-division which had been previously retarded will begin, and the successive stages may be followed.

Though the main points in the process of division may be observed in fresh material, the minute details will be better seen in material which has been fixed in 1 per cent. chromic acid for about four hours, or in picric acid: after washing carefully, the material may be stained with carmine or hæmatoxylin, and mounted in very weak glycerine.

The following points in the process should be specially noted—

1. The disappearance of the nucleolus.
2. The formation of the striated nuclear spindle and of the equatorial nuclear disk.
3. Division of the chromatin which constitutes the disk, and the collection of the two halves at the poles of the spindle as the new nuclei, which are still connected by fine threads.
4. The subsequent dilatation of the spindle, the threads becoming more curved, while from it new connecting threads pass to the peripheral protoplasm.
5. Meanwhile microsomata collect at the equator of the dividing cell.
6. Note also the involution of the chromatophores and their subsequent division.

7. The gradual formation of the septum, beginning at the periphery and proceeding towards the centre.

III. In summer or autumn the process of **conjugation** and formation of the zygospores may frequently be observed in *Spirogyra:* filaments which are about to conjugate assume a position parallel to one another, and on them the following observations are to be made—

1. Cells opposite one another put out rounded processes, which meet.

2. The wall at the point of junction is absorbed, and thus the canal of communication is formed.

3. Meanwhile the protoplasm of the two cells has rounded off, one (the male) usually doing so earlier than the other (the female).

4. The protoplasm of the male cell passes through the canal, and coalesces with the female to form the **zygote.**

5. The zygote surrounds itself with a thick stratified cell-wall, which is smooth or shows various markings of the surface according to species.

By fixing specimens in course of conjugation and staining with hæmatoxylin, Schmitz has been able to observe that the nuclei of the conjugating cells coalesce to form the single nucleus of the zygospore, but according to recent observations this coalescence only takes place after a considerable time, when the zygospore is nearly mature.

For comparison with *Spirogyra* observations should be made on *Mesocarpus*, a filamentous fresh-water Alga, having in each cell a single straight flattened chromatophore, in a central position. The conjugation differs from that of *Spirogyra* in two points: (1) that the

zygospore is formed in the conjugation-canal, and not in the cavity of one of the conjugating cells; (2) that a part only of the protoplasm of the conjugating cells is used up in forming the zygospore.

DESMIDIEÆ

Members of the closely-allied group of the *Desmidieæ* should alse be examined; *e.g.* species of *Closterium*, which is not uncommonly to be found in standing pools of fresh water. Mount specimens in fresh water, and examine under a high power: note—

1. The more or less semilunar curved form of this unicellular Alga.
2. The smooth cell-wall, often marked by transverse striæ, about the equatorial part of the cell.
3. The colourless protoplasmic basis, which includes—
   a. Two large **chromatophores** of equal size, disposed symmetrically on either side of a central clear space.
   b. A single **nucleus,** which occupies the central space, but is sometimes difficult to observe owing to the fact that the chromatophores often extend across the central space, and may even be connected.

Observe further the peculiar plaited form of the chromatophores, which also include several disk-shaped, highly refractive bodies (**pyrenoids**). Stain with an iodine solution: this will make the nucleus more apparent, and the pyrenoids will stain a dusky purple.

The plant multiplies by cell-division: try to observe cells in which the process is going on.

It has recently been shown that the cell-wall of most of the Desmids is composed of two parts which fit together like a pill-box and its lid: it is even stated that in *Closterium* it consists of four parts, there being two external shells, and two girdles which fit within them, about the equatorial position: this is the explanation of the transverse striæ above noted, but it requires high powers to recognize these striæ with certainty as the margins of the shells and girdles above alluded to. In most of the Desmideæ there is a gelatinous sheath, of greater or less extent, covering the cell-wall externally, similar to that in *Spirogyra*.

Other Desmids may also be compared, *e.g. Micrasterias*, which is to be found in peaty pools: it is of flattened form with a deep median constriction, and stellate outline: the pyrenoids are very well seen in this species. For description of other forms of this family, many of which are of great beauty, reference must be made to books dealing specially with the subject: it should be noted, however, in examining them that in the larger majority of them the cell is divided into two symmetrical halves, and that frequently there is a sharply marked equatorial constriction, as in *Micrasterias*.

Conjugation takes place in the Desmids, and it may frequently be observed in *Closterium*, the main points being as follows:—two cells approach one another, usually placing themselves with their longer axes parallel, but sometimes at right angles. Processes are then formed from the two cells, as in *Spirogyra*, which meet, and fusion takes place at the point of contact. Meanwhile the protoplasm passes from the ends of the two cells towards their equators, coalesces, and the **zygote** is formed in central position: it is an oblong cell, which finally becomes rounded, and covered by a thick cell-wall, while the tests of the original cells fall away.

DIATOMACEÆ

Observations should also be made on some of the *Diatomaceæ*, a family of somewhat doubtful affinity, and remarkable for the variety and beauty of form of its members.

Almost any sediment from a fresh-water pool, mounted on a slide and examined under a high power, will show specimens of *Navicula:* it is to be recognized as a unicellular organism pointed at both ends, and showing active movement. Note the yellowish brown colour characteristic of Diatoms, and observe carefully that within the cell-wall there is a basis of colourless protoplasm, in which are embedded a nucleus, and a yellowish brown coloured chromatophore, of variable shape and position.

Specimens of *Pinnularia*, which is also a common form, should be observed, since in it the peculiar structure of the cell-wall is to be seen: it is composed of two halves, **tests,** which fit one inside the other like a pill-box and its lid: this structure is characteristic of the Diatoms, and evidence of it may be found in the various forms examined: but it is only under high powers that the peculiarity can be clearly made out. Compare the structures above noted in the Desmids.

A third form, *Gomphonema*, is to be found attached by a thin transparent stalk to the surface of almost any submerged plant: in this the characters above noted may be again seen.

Attention is to be paid to the varieties of surface-marking in the cell-walls of these and other Diatoms:

for further details and description of other forms reference is to be made to special treatises.

When cell-division takes place in Diatoms, new tests are formed, fitting within the old ones: specimens dividing, or which have recently divided, should be looked for, and examined minutely, under high powers, in order to verify this point.

As the tests once formed do not grow, it is obvious that repeated division will lead to a diminution of size: this is met by the formation of **auxospores,** which may be observed in various Diatoms, especially in summer. There are various details of the process in different Diatoms, but the essential point is that the tests are thrown off, and the contents elongate greatly, and ultimately forming new tests, enter again on a course of division.

The tests are silicified, and by treatment with a little potassium chlorate and nitric acid, and subsequent ignition on platinum foil, skeletons of silica may be prepared, which retain in minutest detail the configuration and surface-markings of the tests of the Diatoms so treated.

CYANOPHYCEÆ

NOSTOC

This plant is to be found growing on turf, or Moss, but not attached to it. It appears as irregular, more or less flattened, olive-green masses, which are brittle when dry, but soft and gelatinous when wetted.

Mount a small piece, or a section of one, in water, and it will be seen under a low power to consist of numerous irregularly coiled filaments, embedded in a gelatinous matrix.

Under a high power each filament may be seen to consist of series of small cells, with granular, blue-green contents, which constitute the great proportion of the filaments; these series are interrupted here and there by larger cells with firm, clearly-marked walls, and transparent contents: these are the **heterocysts.** Observe cases of cells which have recently undergone division. Attempts may be made, by cultivating *Nostoc* in water, to observe the separation and further development of new masses by means of the **hormogonia:** these are short pieces of filaments which escape freely into the water; after a period of movement these settle, form a gelatinous sheath, and grow into a new *Nostoc* mass.

Compare the above observations with those on *Collema* (see below): it is to be noted that *Nostoc* and

Collema are frequently to be found closely associated together, so that it is difficult to tell where the Alga ends and the Lichen begins.

OSCILLATORIA

Organisms belonging to this genus are to be found as dark blue-green, olive-green, or black coverings on damp walls where water is constantly trickling, on wet soil, &c. Mount a small quantity in water, and examine under a high power: they will appear as fine cylindrical, unbranched filaments, with very delicate cell-walls, and cell-contents of various blue, green, and olive tints. Note the septa dividing the whole into disk-shaped cells: also the convex ends, and the constant oscillating movements, from which they derive their name.

Staining with the usual reagents will disclose no nuclei, though minute granules may be stained.

GLŒOCAPSA

This organism may be found, usually associated with others, in those slimy masses which frequently grow on the inside of the glass in hot-houses. Examine it under the microscope, and note the numerous cells with pinkish cell-contents, and much swollen cell-walls. The cells will usually be associated in groups, which, from the course of the lines of stratification of the cell-walls, may be seen to have had a common origin.

The slimy masses of *Glœocapsa* will rarely be found to consist of it alone; other organisms being almost always mixed with it.

FUNGI

I. BASIDIOMYCETES

AGARICUS CAMPESTRIS, *L.* (The Common Mushroom)

I. Examine a brick of "mushroom spawn," such as is sold in the shops for the artificial culture of the Mushroom (*Agaricus campestris*). It will be found to consist of a compost of dried cows'-dung, loam, and clay, in which numerous very fine microscopic filaments are present, or irregularly branched white bands which may be easily recognized with the naked eye: this is the **mycelium.** The best spawn is that in which the mycelium is generally distributed, so as to give the whole mass a uniformly grey appearance. It is to be remembered that the mycelium of other Fungi may, and most probably will, be present in greater or less amount.

Tease out with needles in water some of the mycelium, mount in water, and examine under a low power: note that the white bands recognized with the naked eye are composed of numerous colourless filaments (**mycelial hyphæ**), associated together in a parallel course, while

here and there single hyphæ diverge from the rest, and ramify through the compost.

II. In order to obtain an actively growing mycelium, bearing "mushrooms," the brick is to be broken into pieces, and these must be buried a few inches deep in a compost of similar nature to that of the bricks: the whole is to be kept moist, at a moderately high temperature, and in the dark: the stoke-hole of a conservatory is well adapted for the cultivation of mushrooms. After a period of a few weeks, the compost will be found to be permeated by a mycelium, similar to that in the brick of "spawn," while numerous mushrooms of various sizes will be found connected with it: such a culture as this will suffice for the study of *Agaricus campestris* in the laboratory.

III. Remove a small piece of the mycelium of an actively growing culture, mount it in water, and observe under a low power that it is similar in its main characteristics to that in the dormant state in the brick of spawn.

Having teased it out carefully with needles, examine it in detail under a high power, and observe—

1. The **hyphæ,** of cylindrical form, and with rounded free ends.

2. The irregular **branching** of the hyphæ.

3. The **septa,** which are transverse, and situated at irregular intervals.

4. Hyphæ may frequently be seen to be incrusted by numerous **rod-like crystals:** these are especially numerous in the dormant mycelium: it is to this, in great measure, that the mycelium owes its chalky white appearance.

By applying the ordinary tests, it may be shown that the incrustation consists of calcium oxalate.

IV. Examine a portion of the mycelium which has begun to produce "mushrooms": with a little care the compost may be entirely removed from considerable tracts of the branched mycelium, and then the relation of the latter to the young mushrooms may be clearly seen. If a series of specimens illustrating the development of the mushroom be examined with the naked eye, the following observations may be made—

1. That the mushrooms arise from the mycelium itself.

2. That they appear first as irregular rounded, or oval, upward growths, of denser texture than the mycelium itself.

3. That on cutting one of the smaller mushrooms longitudinally it appears to the naked eye to be of homogeneous structure.

4. That older mushrooms acquire an enlarged head (the **pileus**), which is supported on a cylindrical stalk (the **stipe**). In this state they are termed "button mushrooms."

5. That as the pileus dilates horizontally, a rupture of a veil of tissue (**velum partiale**) about its lower margin exposes a complicated laminated structure formed internally (the **gills,** or **hymenial lamellæ**).

6. Note further the ring or **annulus,** which remains persistent on the stipe of the mature mushroom, and marks the line of rupture of the velum: the corresponding, irregular fringe at the margin of the pileus is also to be recognized.

7. Removing the mature pileus, examine its lower

surface, and note the radiating, more or less darkly coloured **lamellæ,** some of which extend the whole way from the margin to the insertion of the stipe, others only a part of that distance.

Lay the pileus of a mature mushroom with its lamellæ downwards on a sheet of white paper for a few hours: on removing it there will be seen on the paper a sort of print of the configuration of the under surface of the pileus, produced by the fall of the minute, dark-coloured **spores**: the white lines of the print correspond to the spaces between the lamellæ.

This may be particularly well seen in the small *Coprinus*, which appears with great constancy on horse-dung after it has been kept at a moderate temperature under a bell-glass for three or four weeks. This fungus shows on a small scale a structure similar to *Agaricus*, and will serve well as a second type, and it is especially useful since it can be obtained at any time of year.

V. In order to study the structure of the mushroom by means of sections, it is a great advantage to harden the material, and the following treatment has been found to produce good results: treat the fresh material for about twenty-four hours with 1 to ·5 per cent. chromic acid; wash with water, and then successively with 50, 70, and 90 per cent. methylated spirit: the tissues will assume a cartilaginous character, which makes it possible to cut fine sections: in preparing large specimens it is an advantage to cut them up into pieces of moderate size, so that the reagents may gain more ready access to the internal parts.

From material thus treated cut longitudinal sections

of the stipe so as to include both peripheral and central tissues: mount in glycerine, and examine first with a low power: observe—

1. The whole is a **spurious tissue,** composed of elongated septate tubes (**hyphæ**), which are closely interwoven.

2. The diameter of the individual hyphæ is less, and they are more closely packed towards the periphery than near the centre of the section.

Examining the sections under a high power it will further be observed—

1. That the hyphæ are branched, while occasionally their endings are to be seen.

2. That they are thin-walled, the transverse or oblique septa being so disposed that the cells are not much longer than broad.

3. Here and there are to be found hyphæ with fewer septa, and highly refractive contents.

4. The protoplasmic contents of the hyphæ which make up the bulk of the tissue are far from being copious, while no single, well-marked nucleus is to be found in the individual cells.

By careful staining it is possible to demonstrate the presence of numerous very small **nuclei** in the protoplasm of the hyphæ. If fresh material be used, the sections may be stained with methyl-green in 1 per cent. acetic acid, washed with 1 per cent. acetic acid, and mounted in weak glycerine and acetic acid; if chromic acid material be used, it should be carefully washed from the acid, and be stained with hæmatoxylin.

VI. Cut transverse sections of the stipe, and, treating as before, observe that the hyphæ appear circular in section, that they are more loosely packed towards the

centre than at the periphery, and that throughout, intercellular spaces are to be recognized.

Observe under a high power that where the section includes one of the septa (which will thus be seen in surface view), a central highly refractive spot is to be seen, which may also be recognized in the septa in longitudinal sections as a slight aggregation about the centre of the septum.

VII. Passing to the pileus of the mature mushroom, cut tangential vertical sections through it in such a way as to traverse the vertical gills at right angles to their surface: great care must be taken that the surfaces of the gills shall not be injured in the process of preparation, otherwise the basidia and spores which project from their surfaces cannot be observed. Mount in glycerine and examine under a low power: the chief bulk of the section will consist of the massive tissues of the pileus, which show little or no differentiation; passing downwards to the lower surface where the **gills** or **lamellæ** have been traversed, the sections of these will be seen as fringe-like projections from the lower surface: occasionally branching of the gill may be recognized.

Examine the sections in detail under a high power: the following observations are to be made—

1. The mass of tissue of the pileus consists of a complicated plexus of much-branched hyphæ, with large intervening spaces: it is composed of short cells, similar in their characteristics to those which compose the stipe: the chief difference lies in their arrangement. This spongy tissue becomes denser about the insertion of the lamellæ.

2. The sections through the lamellæ show a differentiation into—

a. The central portion (**trama**), in which the septate hyphal filaments are easily recognized running longitudinally down the middle of each lamella, and curving outwards at their ends towards the free surface.

b. The **sub-hymenial layer,** composed of shorter, closely-packed cells, constituting a pseudo-parenchyma: it may, however, be recognized, and especially in sections of young mushrooms, that this pseudo-parenchyma consists of the short-celled, terminal parts of the hyphal filaments which compose the trama.

c. The **hymenial layer,** consisting of oblong, closely packed cells, having their longer axes perpendicular to the outer surface: of these cells two types are to be distinguished—

i. The **paraphyses,** which are somewhat narrower and have smooth rounded ends.

ii. The **basidia,** which are more bulky, and longer: each bears on its end **two fine processes** (**sterigmata**); at the extreme tip of each of these there appears a swelling which develops into the mature **spore.** Note various stages of development of the sterigmata, and spores.

VIII. Remove a whole gill carefully from a fresh mushroom, mount it on a slide, without any reagent or cover-slip, and examine its surface with a medium power: it may then be seen that the dark colour is due to the dusky spores, which are thickly distributed over the surface of the gill in pairs, **two being pro-**

duced from each basidium: note further the pale colour of the tissue of the hymenium, and the rounded ends of the paraphyses, and of those basidia which are young, or have already produced mature spores.

Attempts should be made to germinate the spores in a decoction of horse- or cow-dung: a very small number of the spores should with a sterilized needle be introduced into a drop of the decoction recently boiled, the drop having been placed in the centre of a cover-slip which had been recently heated. The cover-slip is then to be inverted and placed over a moist chamber constructed as described in Appendix A. The spores can be examined at intervals under the microscope, and the germination followed.

A comparison may be made with *Coprinus micaceus:* this Fungus appears almost with certainty if cow-dung be kept for three or four weeks at a moderate temperature under a bell-glass: if sections be cut from the lamellæ, the structure of the hymenium will appear similar to that above described, but the number of sterigmata on each basidium is **four.** On species of *Coprinus* large bladder-like cells (**cystidia**) are found projecting from the hymenial layer.

Examine specimens of other Hymenomycetous Fungi, *e.g.* species of *Polyporus, Dædalea, Boletus,* and *Hydnum:* note in them the difference of conformation of the thallus, and especially of the hymenial surface; also the difference of texture: thus in *Dædalea quercina,* and in some species of *Polyporus,* the thallus is hard, and of a corky or woody nature, while in others (*e.g. Polyporus giganteus*) it is soft and succulent.

Sections should be cut from the thallus of *Dædalea quercina,* or some other woody form: an examination of them will show that, though the thallus is hard, the structure of it is similar to that of the mushroom, the whole being composed of branched septate hyphæ.

Sections should also be made through the hymenium of some species of *Polyporus* in such a way as to cut the pores transversely : examination of these will show the hymenial layer lining the pores, and consisting, as in the mushroom, of paraphyses and basidia, the latter producing sterigmata, and spores ; but in *Polyporus* the hymenial layer is less closely packed and regular.

A comparison should also be made of some types of the Gasteromycetes, *e.g. Lycoperdon, Geaster, Crucibulum*, and *Phallus*. In these forms it will be seen that there is considerable variety in detail of arrangement of the hyphæ, resulting in a marked difference of external conformation of the mature Fungus. Still, sections of them in relatively young stages will show that they also are composed of branched and septate hyphæ, while the mode of formation of the spores on the basidia of more or less regular internal hymenial layers, corresponds essentially to that already described for the mushroom.

Dacryomyces may also be examined as an example of the Tremellini. It appears as gelatinous orange masses on rotting wood, especially of the Pine. Sections of it will show the deliquescent nature of the fungus, the hyphæ having a gelatinous outer layer of the cell-wall. The spores, borne in pairs upon basidia, may be seen at the surface of the thallus.

II. ÆCIDIOMYCETES

PUCCINIA GRAMINIS (Æcidium Berberidis), Rust of Wheat

A. *Puccinia Stage*

I. There may often be found on the stems and leaves of wheat and others of the Gramineæ in winter, dark oblong patches, which owe their origin to a Fungus (*Puccinia graminis*) that infests the tissues, and produces the disease called *Rust*.

Examine one of these patches with a lens, and note that the superficial tissues of the wheat are ruptured by a longitudinal slit, and the torn edges are turned back, so as to expose a dense, dark-coloured mass, which protrudes from within: the nature of this mass must be studied by means of sections.

II. Cut transverse sections of the leaf-sheath, or other diseased part of the Grass plant, taking care that the section shall traverse one or more of the dark patches of Rust: mount in glycerine, and examine under a low power. Observe that the structure of the greater part of the section is normal (see page 175, &c.): the dark patches will be seen to be opposite one of the spaces

between the vascular bundles, while the epidermis, which normally covers over the tissues, is ruptured. In case it is the leaf which has been cut, dark patches may be observed as rupturing and projecting through the epidermis of both the upper and lower surfaces.

Put on a high power, and in a thin section observe—

1. The thin **hyphæ** of the branched **mycelium** of the parasite (*Puccinia*), which ramify in the softer, succulent tissues, but do not as a rule attack the sclerenchyma, or vascular bundles: they may be traced up to the dark patches above noted.

2. The masses of dark brown **teleutospores** or **winter spores,** which are produced by this mycelium, each spore being borne on a thin pedicel: each consists of two cells, with thick walls, differentiated into two layers, the **exospore** and the **endospore.** In the protoplasmic contents of each cell is a clear spherical body, which may be the nucleus, but this is not certain.

III. If pieces of a Grass plant bearing teleutospores be kept in a moist atmosphere (on wet blotting-paper, under a bell-glass) in the spring-time, a fine, white, semi-transparent growth will be produced from the teleutospores: this is the **promycelium.** Remove some of these germinated teleutospores carefully with a needle, and mount in water: if this be done without injuring the promycelium, it will be seen under a high power that one or both of the cells of the teleutospore have put out a germinal tube (the **promycelium**) by rupture of the exospore, and protrusion of the endospore: this promycelium divides into four

or five cells, each of which (excepting the basal one) produces a conical process (the **sterigma**): the end of each of the sterigmata swells into a small irregularly roundish body (the **sporidium**), which ultimately becomes detached.

By the above simple method of preparation the promycelium is apt to be damaged; it is much better to remove the teleutospores before germination, and to cultivate them in a hanging drop of water on the slide (see Appendix A.) At the right period of the year, the germination takes place in about twenty-four hours: if this succeed, the additional advantage is gained from cultivation on the slide, that the observer will be able to follow the successive stages of the germination in an individual specimen, by repeated examination of it at short intervals of time.

It is known that the sporidia thus produced are not capable of further development on the Grass plant: this only takes place when they gain access to the Barberry (any species of *Berberis*), or other plants of allied genera, *e.g.*, *Mahonia*. In order to acquire evidence on this point, cultures should be prepared as follows; students must, however, be prepared for disappointment owing to various technical difficulties, which will only be duly appreciated in practice:—Keep fresh, young leaves of *Berberis* in moist air under a bell-glass, and in drops of water placed on the surface of them immerse some teleutospores which are known to be in a fit condition for germination: after an interval more than sufficient for complete formation of the sporidia (and De Bary states that infection may occur in twenty-four hours), strip off a small piece of the epidermis, or cut tangential sections from where the drop was placed: mount in water with the outer surface of the epidermis uppermost, taking care to avoid pressure by the cover-slip. Examine under a high power to observe the mode of germination of the sporidia, and the penetration of the germinal tube **through the outer wall** into a cell of the epidermis, whence it proceeds to the mesophyll, and, branching, spreads through considerable tracts of tissue: the result of this may now be studied in the mature condition on the leaf of *Berberis*.

B. *Æcidium Stage.*

IV. Note in early summer on the leaves of *Berberis* irregular bright yellow blotches, the tissues of the leaf appearing swollen at those spots, and projecting convexly on the lower surface, while the upper surface of the blotch is usually concave: on the lower surface numerous irregularly distributed yellow cups (**æcidium cups**) may be seen projecting slightly beyond the surface, while on the upper surface also may be seen projecting organs of smaller size, and irregular distribution (**spermogonia**).

V. Cut transverse sections of a diseased leaf, so as to pass through one of these blotches: mount some in glycerine, others in chlor-zinc-iodine, and examine under a low power: observe—

1. In the thinner **normal** part of the section, that between the upper and lower epidermal layers there is a mesophyll consisting of a single palisade layer, and five or six irregular layers of spongy parenchyma.

2. That the greater bulk of the **infected** part is due not so much to increased number of the cells as to the larger size of the individual cells and of the intercellular spaces.

In the sections prepared with chlor-zinc-iodine, if a good staining has been effected, note with a low power that the fungal tissues are but slightly stained yellow, while the tissues of the host are stained in the usual way, chiefly a dark blue. Recognize as the most prominent parts of the parasite—

1. The **æcidia,** cup-like structures, containing a

closely packed mass of **spores,** and opening by rupture through the lower epidermis of the host.

2. The **spermogonia,** relatively small, flask-shaped organs opening on the upper surface of the leaf.

Having thus gained a general idea of the sections, examine them in detail under a high power, and note that in the infected patch the cells of the host are apparently embedded in a felt of **mycelium,** consisting of septate and branched **hyphæ,** which traverse and completely choke up the intercellular spaces: they are but slightly stained with chlor-zinc-iodine, while the cell-walls of the host plant assume a dark colour: they are for the most part confined to the intercellular spaces, and especially those round about the æcidia; but it is stated that occasionally they penetrate the cells of the host, and though this is not easy to see, examples of it should be looked for. Turning to the æcidium observe—

1. Its cup-like form.

2. The dense felt of hyphæ at the base of it.

3. Immediately above this is the **hymenium,** a layer composed of closely packed, parallel, rod-like cells (**basidia**), arranged perpendicularly to the outer surface of the leaf.

4. The rows of **spores,** which have been successively abstricted from the basidia: observe the hexagonal form, thickened wall, and orange colour of the spores, and the way in which the spores of contiguous rows fit together.

5. The **peridium,** consisting of a single layer of cells enveloping the mass of spores: the form and arrangement of the cells resemble that of the spores themselves

though not so regular: note the thickened and striated outer wall.

Prepare similar sections from a young blotch, in which all the æcidia have not yet ruptured the epidermis of the host: and note—

1. The origin of the æcidia in the mesophyll of the host.
2. That the æcidia are relatively narrow when young, the hymenium growing broader as it grows older, by intercalation of new basidia.
3. The traces of displacement, and ultimately of rupture of the superficial tissues of the host.
4. The outgrowth of the peridium as a tube open at the apex, and extending beyond the general surface of the *Berberis* leaf.

Returning to the **spermogonia,** observe—

1. The closely packed, parallel, rod-like hyphæ converging to the centre (**sterigmata**).
2. The minute oval bodies (**spermatia**) abstricted from them, and escaping through the narrow pore on to the outer surface of the leaf.
3. The brush of hyphæ which protrude through the narrow pore.

A careful teasing out of the spermogonia with needles, and examination under a high power will be a profitable exercise. Attempts may also be made, with suitable precautions, to cultivate the spermatia in various nutritive solutions.

VI. It is known that the æcidium-spores of this fungus will not infect the Barberry plant afresh, but will only germinate so as to infect a Grass plant; thus the fungus is an example of "Heterœcism." The spores retain their germinating power only for a short period.

Take some fresh spores from an æcidium, and place them in a drop of water on the surface of a fresh leaf of some Gramineous plant: after keeping it in moist air for about 48—60 hours, strip off a part of the epidermis, or better, cut tangential sections of that part on which the spores have been placed: mount in water with the outer surface of the epidermis uppermost, and examine under a medium power: observe that the **æcidium spores** have produced tubular **hyphæ,** which make their way, **through the pores of the stomata,** into the tissues of the Grass plant.

VII. Infect a Grass plant with **æcidium spores** and keep it in a moist atmosphere: in about a week reddish swellings will appear about the points infected, and the epidermis will be ruptured.

Cut transverse sections so as to traverse one of these ruptured spots: mount in water, and observe under a medium power: note—

1. The branched mycelium ramifying in the tissue of the Grass.

2. The ruptured epidermis.

3. The closely packed **uredo-spores** of simple oval form, borne on thin pedicels (basidia). Observe further the **exospore,** rough with small outgrowths: the **endospore,** with four germinal pores, arranged equatorially; here the inner wall is wanting: note the protoplasmic contents with reddish granules.

Attempts should be made, as above directed for the æcidium spores, to infect leaves of the Barberry and of the Grass with these **uredo-spores,** when the infection will be found to succeedon the Grass, but not on the Barberry.

The infected Grass plants which have produced

uredo-spores should be kept till the autumn, when the patches which before produced uredo-spores only will, on investigation as above directed, be found to bear **teleutospores** intermixed with them, and finally to assume the winter condition of containing **teleutospores** or **winter-spores** only, in which condition the winter is passed: with this stage the study of the Fungus, as above directed, was begun.

III. ASCOMYCETES

A. *DISCOMYCETES*

PEZIZA

For comparison on the one hand with *Claviceps* (p. 481) and *Eurotium* (p. 484) as members of the Ascomycetes, and on the other with the Lichens to be described below (p. 473), observations should be made on some species of *Peziza*. This is a very large genus, and the specific differences are somewhat difficult to master, but any species will serve the present purpose. It has been shown that some species at least have a gonidium-bearing stage, thus the mould known as *Botrytis cinerea*, which is common on decaying leaves, has been proved to be a stage in the life-history of *Peziza Fuckeliana*; but it is with the mature fructification or **apothecium** that we shall have to deal first.

The flat or cup-shaped fructifications of *Peziza* are commonly to be found growing on decaying wood, &c., and vary greatly in size and colouring in different species. *Peziza coccinea* is a species which is conspicuous in woods in early spring as a carmine coloured cup about

one inch in diameter, attached by a short stalk to rotting sticks.

P. stercorea, is an orange-coloured one very common on cow-dung. Having collected some, observe with the naked eye—

1. The smooth upper surface or **hymenium**.

2. The margin, which is hardly developed as a distinct excipulum.

3. The lower surface and base, which is usually covered with fine hyphal filaments, serving as rhizoids.

From material, either quite fresh, or hardened in alcohol, cut median vertical sections: mount in glycerine, and observe—

1. The large-celled pseudo-parenchyma about the lower surface, with the projecting rhizoid-like hyphæ which penetrate the substratum.

2. Passing upwards through the section the hyphal character becomes more obvious, the branched filaments forming a dense plexus.

3. The tissue again assumes more of a pseudo-parenchymatous nature in the **subhymenial layer.**

4. The **hymenium,** which is similar in its character to that of *Parmelia*, consisting of—

a. Elongated, narrow **paraphyses,** and—

b. Wider **asci,** each of which contains eight **spores.**

A careful comparison of various asci will give some idea of the mode of development of the spores by a process of free-cell formation.

Other forms of Ascomycetous Fungi may also be compared with advantage; *e.g. Morchella* (the Morel), in which the surface of the irregularly convoluted head is covered by the hymenium, similar to that of *Peziza*.

Observations should also be made on *Botrytis cinerea*, an ashy grey mould which is common on decaying vegetable matter, and even on parts of living plants in greenhouses which are kept too damp: this is the conidial form of a *Peziza*. Remove a small quantity of the mould, moisten it with a drop of alcohol, and mount in water: the **conidiophores** are upright-growing septate and branched hyphæ, which bear the **conidia** in tufts, on the end of the main hyphæ, or on short lateral branchlets, which may again bear secondary branches. If old conidiophores be examined, they may be found covered with the scars where lateral branchlets had previously been; these, after producing their conidia, shrivel up, and the scar is all that remains of them, while the conidiophore may continue its growth and produce fresh branchlets and fresh conidia.

Note the position of the conidia, and the delicate **sterigmata** by which they are attached.

The conidia may be readily germinated in Must, or in a freshly boiled decoction of French Plums, and cultures of the mycelium, which shows many interesting features, may be thus obtained. If a pure culture be desired the watch-glass, the decoction, and all the needles, &c., which are used must be carefully sterilized by heating, and a very few conidia must be taken from a perfectly pure patch of the fungus.

The fungus *Ascobolus* is also to be examined. It appears with great constancy on horse- or cow-dung, if kept for two or three weeks under a bell-glass. If the small flat cups be cut vertically, sections will show a structure essentially similar to *Peziza*, but on a smaller scale.

In sections of the fruit-body when very young the archicarp and antheridial branch should be looked for, as well as their connection with the ascogenous hyphæ.

B. *LICHENS*

PARMELIA PARIETINA (Yellow Lichen)

I. This species is very commonly to be found growing on tree-trunks, walls, roofs, &c.: note its **foliaceous** thallus, which has no definite mode of branching, and is closely applied to the substratum. It is of a bright olive-yellow colour on the upper surface, but white underneath: note the processes from the under surface (**rhizines**) by which it is attached to the substratum: also its brittle character when dry: soak it in water, and it will become soft but leathery.

On the upper surface observe the more or less flattened or cup-shaped **apothecia,** which are of darker colour than the rest of the thallus, and attain a diameter of about one-eighth of an inch: note that those near the margin of the thallus are smaller than those nearer the centre.

II. From material which has been kept dry, select a small part of a thallus where no apothecia are present: soak it for a short time in alcohol, and embed in paraffin: cut as thin sections as possible: having allowed them to swell as much as they will in water, mount them in glycerine, and examine under a low power: observe—

1. The irregular outline of the section.

2. The greater part of the thallus made up of colourless transparent tissue (**fungal tissue**).

3. The green **gonidial layer,** which is not very regular or sharply limited, and is situated at some distance below the upper surface of the thallus: since the gonidia are confined to a certain region of the thallus, and not distributed uniformly through it, this may be distinguished as a **heteromerous** Lichen.

4. The **rhizines,** which may be seen as irregular brushes of colourless tissue projecting from the lower surface.

Examine the thinnest section on the slide under a high power, and passing successively from the upper to the lower surface, observe—

1. The upper broad band of pseudo-parenchyma, the outer limiting part of which is coloured yellow: the cell-walls of this tissue are swollen, and the cell-contents not voluminous or obvious: this band passes without any clearly marked limit into—

2. The **gonidial layer,** where may be seen, embedded in the rather more lax, colourless, fungal tissue, round cells with definite cell-walls, and green-coloured contents—the **gonidia,** or Algal constituents of the Lichen thallus.

3. Passing downwards, the colourless tissue of the thallus becomes more lax, and has a distinctly **hyphal** character, betraying more clearly here its Fungal characters. Note carefully how the hyphæ come into very close communication with the gonidia.

4. Proceeding to the lower limit of the section, a second band of pseudo-parenchyma is reached, which is colourless, but otherwise similar to the upper.

For comparison with the above type, other Lichens should also be examined: for instance, *Usnea barbata*, which is frequently to be found growing on the bark of trees in hilly districts: it is a **fruticose** Lichen, with a cylindrical, much branched, grey thallus, which bears near the tips of the branches the disk-shaped **apothecia.** Cut transverse sections of the thallus and treat as before: observe—

1. The irregular outline of the nearly circular section.
2. The broad band of external pseudo-parenchyma.
3. The gonidial layer as in *Parmelia.*
4. A broad, and very lax hyphal band.
5. A central dense strand of pseudo-parenchyma with thick swollen walls.

Thus *Usnea* is again an example of a heteromerous Lichen, but of the fruticose type.

Examine the gelatinous thallus of *Collema pulposum*, or other species, which may be found growing on moist soil, stones, &c. The foliaceous thallus is somewhat thick and bulky, and of a gelatinous consistency when moist; when dry it is relatively thin, and brittle. It is well to observe the changes which moistening produces on dry specimens.

Cut sections through the dry thallus, soak them in water, and mount in water, or in weak glycerine: examine under a high power: it will then be seen that the thallus consists of—

1. A gelatinous transparent matrix, similar to that of *Nostoc* (see page 451).

2. Chaplets of cells coloured greenish-blue (**algal cells**), occasionally interrupted by larger cells, with thicker walls and no green colour (**heterocysts**).

3. Branched and colourless **fungal hyphæ.**

Note that the Algal and the Fungal constituents are distributed uniformly throughout the thallus, the Alga not being restricted to a definite zone: this is thus an example of the **homoömerous** Lichens.

III. Some specimens of *Parmelia* will be found to show more closely packed convolutions than others, and on these it may be noted that **apothecia** are few, or entirely absent: it is on these that the **soredia** are more especially to be sought for. Soak such a specimen (which has previously been kept dry) in water: then, having dried off the excess of water with blotting-paper, press its upper surface on a glass slide, when, on removing it again, a sediment will be left in the water on the slide: mount this in a drop of glycerine, warm gently, and examine under a high power. Various objects which have no relation to the thallus will be seen, such as grits, various Algæ, &c., &c. Amongst these will be seen roundish bodies (**soredia**) of various size and complexity, composed of the same constituents as the thallus, viz.—

a. Fungal hyphæ, enveloping, and completely inclosing—

b. The gonidia, of which one or more may be present in each soredium.

Attempts should be made to grow Lichens from the soredia: pieces of porous tile should be heated to kill other organisms, then saturated with water, and the soredia sown in small numbers on their surface.

In some other Lichens the soredia are produced in a more prominent manner than in *Parmelia*: thus in *Usnea* and *Cladonia* they may be recognized as a powdery covering of some parts of the thallus, and are especially obvious after rain. In the gelatinous Lichens soredia are absent, but the thallus may be reproduced by the outgrowth and ultimate abstriction of processes consisting of both Algal and Fungal constituents: these may often be seen projecting from the outer surface in sections of these Lichens.

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IV. Having carefully noted the form of the apothecium of *Parmelia*, and its attachment to the thallus, cut thin vertical sections through it, and, mounting in glycerine without previously allowing them to swell in water, observe, under a low power—

1. That the structure of the part of the thallus which bears the apothecia is similar to that described above.

2. That the lower stratum of the tissue composing the cup is similar to the above, but note especially the very considerable masses of gonidia immediately below the upper stratum of the apothecium.

3. That the upper stratum of the apothecium consists of closely packed, more or less club-shaped elements, regularly arranged perpendicularly to the surface: this is the **hymenium,** of which two constituents are to be distinguished—

a. The **asci,** which may be recognized as relatively wide, club-shaped cells; the contents vary according to the stage of development: when mature each contains eight oval **spores.** Note that various stages of development are to be seen in the same apothecium.

b. The **paraphyses,** which are relatively narrow, and take no direct part in reproduction.

Treat a fresh section through an apothecium with iodine solution, and note that the cell-walls of the hymenium assume a **blue coloration.**

Treat some sections through an apothecium with water, and observe how greatly the hymenium swells, so that it is thrown into numerous folds: it may be remembered that this property of swelling of the constituents of the hymenium is connected with the rupture and extrusion of the spores when mature; since the

margin of the apothecium (the **excipulum**), consisting of less swelling tissue, resists the increase of bulk of the hymenium, and pressure is thus established.

Note that the ends of the paraphyses and of the older asci have a yellowish-brown colour, similar to that of the surface of the rest of the thallus: treat sections with potash solution and warm: the parts coloured yellow assume a pink colour, which diffuses out into the solution, and also into other tissues of the thallus: neutralize with acetic acid, the colour will disappear, but may be again produced by adding potash. Compare with this the well-known properties of **litmus**, which is produced from *Roccella tinctoria* and other Lichens.

V. Transverse sections may also be cut through the hymenium: treat as before, and note the **asci**, which appear of circular outline, of relatively large size, and contain the highly refractive spores: the **paraphyses**, of relatively small bulk, are closely packed round them.

VI. Observe the spores in detail: this may be done by inverting the surface of an apothecium (previously kept dry) in a drop of water on a slide, when spores will be ejected, by means of the pressure due to swelling as above noted: mount these in water and examine them under a high power: observe their oval form, and the presence of a highly refractive body at either end, the two being connected by a fine strand: stain with an iodine solution, the highly refractive bodies as well as the connecting strand will stain yellowish-brown (protoplasmic body), the rest of the spore is not distinctly stained (cell-wall).

There is considerable variety in the number of the spores produced in each ascus in various Lichens, and they attain in some cases great complexity of structure, as well as considerable size.

Compare *Megalospora*, where in each ascus only one large

unicellular spore is formed ; *Pertusaria*, with two, four, or six, in different species ; *Peltigera*, *Graphis*, and certain species of *Collema*, in which each spore is composed of four or more cells **(sporæ compositæ)**.

VII. The discovery of the sexuality in certain of the Lichens is one of the most marked observations of recent years, and attempts should be made to see at least the more important organs concerned in the process, which are on the one hand the **spermogonia** (male), on the other the **ascogones** (female): these may both be seen in species of *Collema* collected in early spring ; while the spermogonia, which in other Lichens are often difficult to find, may be observed in any specimen of "Iceland Moss" (*Cetraria Islandica*) as sold in druggists' shops.

Observe on specimens of *Cetraria* a fine fringe of minute teeth along the margin, each of these contains a **spermogonium** at its apex: embed a portion of a thallus bearing these teeth, and cut fine transverse sections of it : the teeth will thus be cut longitudinally. Mount in glycerine, having previously allowed the sections to swell in water, and look over the sections under a low power to find examples of spermogonia cut longitudinally, which will appear as flask-like cavities. Having found one of these, examine it under a high power and observe—

1. The general structure of the thallus corresponding to the **heteromerous** type above described (*Parmelia*).

2. The hollow **spermogonium,** composed exclusively of the Fungal constituent of the Lichen, the hyphæ being closely packed and pointing radially inwards to the

cavity, and giving off at their apices by abstriction the oval, motionless **spermatia,** which escape by a fine **ostiole.**

The escape of the spermatia may be observed in fresh material, by mounting a piece of the thallus bearing spermogonia in a drop of water, after it has previously been kept dry for a time: the spermatia are then extruded through the ostiole, embedded in a gelatinous matrix. (Compare these spermogonia with those of *Puccinia.*)

VIII. Though the search for the female sexual organs which precede the formation of the apothecia has been successful only in few cases, still there is no great difficulty in observing them in the genus *Collema.* Material for this purpose should be collected in early spring (March), and sections may be cut from it fresh, or better after hardening in alcohol. Cut fine sections from a part of a thallus which bears as yet no apothecia, let them swell in water, or weak glycerine, and mount in glycerine: observe here and there a fungal filament to have become coiled at some distance below the surface, and then to be continued almost directly to the outer surface of the thallus, beyond which it projects—this coiled part is the **ascogone,** while the straight part is the **trichogyne.** Occasionally spermatia are to be seen attached to the apex of the trichogyne.

In other sections apothecia may be seen in various stages of development, or spermogonia having a structure similar to that above described for *Cetraria.*

C. *PYRENOMYCETES*

CLAVICEPS PURPUREA (Ergot)

I. This Fungus is found infesting the ears of various Grasses: it is very prevalent on the Rye, and is commonly styled the Ergot of Rye; but it is also to be seen on other Grasses, *e.g.* on *Lolium perenne*, *Glyceria fluitans*, &c.

Examine specimens of ergotised Grasses taken in the dormant condition in autumn or winter: the arrangement of the parts will be found to be normal, but often, in place of the normal ovary, there may be seen an enlarged, hard, dark-purple body, which is easily detached: this is the **sclerotium** of the Fungus. Note at the apex of the sclerotium a lighter coloured, easily detached body: this is all that remains of the **gonidiophore** (Sphacelia form) which is produced in early summer, and is now dry and shrivelled.

II. Cut transverse sections of the sclerotium in the dormant winter condition: mount in glycerine, and observe under a low power—

1. The irregular outline of the section, and compare this with the whole sclerotium, which is often marked by longitudinal grooves or slits.
2. The external dark-purple covering.
3. The internal, dense white mass.

Examine a fine section closely, under a high power: it will then be seen that the whole is composed of a **pseudo-parenchymatous tissue**, with relatively thick cell-walls, and abundant oil stored in the cells: this collects in large globules on and about the

sections. It will be further observed that the peripheral tissue is similar to the central, with the exception of colour and consistency. Small masses of tissue of the ovary of the Grass may sometimes be found embedded in the sclerotium.

Treat a section with chlor-zinc-iodine: the cell-walls will stain a faint yellow, and this colour is not changed even after some hours' treatment with the reagent (fungal cellulose).

III. Set sclerotia to germinate, half buried in moist clean sand, at a moderate temperature: this will succeed best in spring or early summer, since even if the cultures be started at other seasons, under most favourable conditions, the sclerotia will (with very few exceptions) undergo no change till the proper season comes round. When germination begins, the peripheral tissue will be broken through at one or more points by the swelling of a light-coloured mass within: this grows quickly, so as to form a **stroma**, which consists when mature of an erect cylindrical **stalk** one inch or less in length, and a spherical **head**. Examine the latter with a lens: it is when mature of a purplish colour, and marked with numerous projecting dots.

IV. Cut transverse sections of a sclerotium which has already germinated, and in such a way as to traverse the base of a mature stroma: mount in glycerine, and observe—

1. That the store of oil, &c., in the sclerotium is much reduced, and the whole tissue soft and apparently exhausted.

2. That the tissue of the stalk is of a distinctly hyphal character, the hyphæ being arranged parallel one to another, and septate.

3. That at the base of the stalk the hyphæ appear to have originated from the pseudo-parenchyma of the sclerotium.

V. Cut median longitudinal sections of the mature head of a stroma, and including the upper part of the stalk: mount as before, and examine under a low power: observe—

1. The upper part of the stalk showing the same structure as the base.

2. The semi-lunar section of the head, inserted upon it.

3. The numerous flask-shaped **perithecia** embedded in its mass, each having a slight papillose projection of the surface opposite it: these have already been observed from outside.

Examine the sections under a high power, especially the head and note—

1. The darker, denser zone immediately above the insertion on the stalk.

2. The more spongy mass of tissue forming the bulk of the head: in this, the hyphæ are more laxly arranged, and thicker: externally will be seen—

3. A more dense, pseudo-parenchymatous peripheral band.

4. The **perithecia** will now be seen to be cavities, each with an **ostiole** which opens at the apex of one of the papillose projections. Note the rather denser coat of tissue lining the cavity, which widens at the base into a **sub-hymenial** mass: this gives off upwards numerous club-shaped **asci**, closely packed, and without paraphyses; these, when mature, contain the peculiar elongated filamentous **spores.**

VI. Attempts should be made, by cultivation of these spores in water, to observe the first stages of germination, when it may be seen that hyphal tubes are formed at a number of points on the single spore.

VII. Attempts should also be made in early summer to infect the inflorescence of various Grasses on which the parasite is known to grow with the fresh spores, and to follow out the development and characters of the **gonidiophore** or **Sphacelia** form of the Fungus.

D. *CLEISTOCARPOUS ASCOMYCETES*

EUROTIUM ASPERGILLUS GLAUCUS

I. Keep a slice of dry bread under a bell-glass, until it becomes mouldy. Even a superficial examination of it will show in most cases that more than one kind of Mould is present. Among the rest the most prominent will probably be one which bears roundish, white or pale green heads closely aggregated, and borne on stalks of about one-sixteenth of an inch in length: this is the conidial form of *Eurotium Aspergillus glaucus*, and the branches bearing the heads are styled the **conidiophores.**

Shake some of these gently with the point of a needle: numerous minute powdery bodies (the **conidia**) will be liberated, and will float away as a fine cloud.

If it be desired to obtain a pure culture of this fungus for further study, the following precautions are to be taken. Thoroughly boil a few French plums till they are quite soft: this will completely sterilize them. Having previously sterilized a plate and bell-glass by exposure to high temperature (boiling-point for a long time, or a higher temperature for a shorter time),

place the plums on the plate, and infect them by transferring a few conidia from as pure a patch of the mould as can be found on the bread : the transfer is to be made with a needle which has previously been heated. If these precautions are taken, and the plums be kept covered, a pure culture of the Fungus should be obtained.

II. From a pure patch of this green Mould remove a small portion with a needle, avoiding mechanical roughness as much as possible : lay it on a slide, moisten with a single drop of alcohol, then add water, and cover gently with a cover-slip. Examine it under a low power, and observe—

1. The stalked **conidiophores,** with large, mop-like heads.

2. The colourless tangled **mycelium** attached to these, and from which they spring.

3. The innumerable detached **conidia** which will be found thickly distributed throughout the preparation.

Having selected one of the largest of the conidiophores, examine it in detail under a high power, noting especially—

1. The robust **stalk,** usually without septa : its wall is clearly defined, and the protoplasmic contents granular and vacuolated.

2. The transversely septate, branched **mycelium,** from which the conidiophores arise as vertically growing branches, usually from a point immediately behind one of the septa : in this as in other cases of branching of the mycelium, the branch grows out at right angles from the hypha which bears it.

3. The swollen spherical **head** of the conidiophore, with its conidia in radiating rows inserted upon it.

Examine carefully the way in which the conidia are produced, noting—

a. The **sterigmata,** which are peg-like radiating outgrowths from the head of the conidiophore.

b. The series of **conidia,** in successive stages of development, which have been successively formed by **abstriction** from the sterigmata.

c. The oval form, and spiny surface of the mature conidium.

In order to observe the successive stages of development of the conidiophore, small portions of the Fungus should be taken from the white patches, where the growth is younger, and be treated as before. In these specimens the following points are to be observed—

1. The conidiophore as a club-shaped thick erect hypha.

2. The swelling of the head, though it at first remains quite smooth.

3. Minute papillar outgrowths appear on the surface of the head—these are the young sterigmata.

4. The sterigmata elongate, and become attenuated at the tips.

5. The successive stages of abstriction of the conidia from the apices of the sterigmata.

III. In order to trace the germination of the conidia, they should be cultivated under microscopic observation on the slide. For this purpose a moist chamber is to be prepared as directed in Appendix A. It will be necessary to take certain precautions to reduce the probability of access of foreign spores to a minimum, and so insure as nearly as possible a pure culture. Prepare a nutritive solution by boiling French plums in water: this

decoction is to be used **very dilute,** and is to be boiled **immediately before starting the culture,** so as to kill any foreign spores which may be already present: with the same object, the glass slide, cover-slip, and needles are all to be heated in a spirit-lamp, and the porous pad for the moist chamber is to be well boiled in water.

Having made these preparations, place a single drop of the dilute, sterilized decoction on the cover-slip: then with a needle, moistened with the sterilized fluid, remove from as pure a tuft of *Eurotium* as can be found a **small number** of conidia, and place them in the single drop on the cover-slip: examine under a low power to see that the number of conidia is small, then quickly invert the cover-slip and place it over the round hole punched in the porous pad. Keep the preparation thus made under a bell-glass, and observe it from time to time under the microscope: if the culture be successful, the successive stages of germination and of further development of the Mould may be watched in detail.

IV. The **perithecia,** and the **archicarps** (female organs) which give rise to them, are to be sought for on a mycelium which has already produced mature conidia: the ripe perithecia (*Eurotium* fruits) may be readily recognized in old cultures on dry bread, as minute yellowish spherical bodies, easily distinguished by the naked eye.

A. Remove a small piece of mycelium which has already borne mature conidiophores, and is thus likely to bear young **archicarps:** moisten it with alcohol, and then wash off in a watch-glass in water as many of the conidia as possible: tease it out with needles,

and, mounting in water, examine under a high power. Observe—

1. That the same mycelium which bears the conidiophores also produces relatively thin whip-like branches, with highly refractive contents.

2. That some of these branches become coiled, at first loosely, but later in a tightly packed spiral of four or five coils, and consisting of several cells: these spirals are the **archicarps.**

3. That first one, and subsequently several hyphal branches appear below the closely coiled archicarp, forming an investment round it: the first formed branch is called the **pollinodium** (male organ), and comes in close contact with the apex of the coiled archicarp.

The actual transmission of substance from the pollinodium to the archicarp has not been seen, but observations point to the disappearance of the membrane separating them, and thus continuity appears to be established between the two protoplasmic bodies.

B. From a culture of some six weeks' duration on dry bread pick off with a needle some of the minute spherical perithecia: mount them in water and examine under a low power: observe—

1. The round or oval form of the perithecia.

2. That they are composed of a small-celled pseudoparenchymatous tissue.

3. Their yellow colour.

4. Their insertion, each being borne on a single filament of mycelium.

The yellow colour is due to an oily substance, which is soluble in alcohol, or in potash solution.

Treat some perithecia with a weak potash solution, mount them in glycerine, and examine under a high power: note—

1. The **wall** of the perithecium, consisting of a single layer of somewhat flattened cells.

2. The cavity surrounded by that wall filled with bodies of oval form—the **asci.**

In order to be able to examine the asci in detail, mount fresh perithecia in glycerine, press with a needle on the cover-slip, so as to burst them, and note—

1. The ruptured wall, as before.

2. The oval **asci,** each of which contains eight **ascospores,** of oval shape when young, and biconvex-lens shaped when mature.

3. Other cells may also be found which belong to the filling-tissue or pseudo-parenchyma; this is derived by ingrowth from the wall of the perithecium, and is only to be found in young perithecia: at the period of maturity it is completely absorbed.

In order to trace the various steps of development of the perithecium, observations should be made at various times during the progress of the culture, and the origin of the asci from the spirally coiled archicarp is to be observed in specimens made transparent with potash and glycerine. Further, the origin of the wall of the perithecium, from branch filaments which grow round and invest the archicarp, is also to be traced, and finally the ingrowth of the wall between the products of the archicarp, so as to form the "filling-tissue."

These points, however, and especially those changes which take place in the later stages of development, are best to be seen in sections cut through the perithecium: these may be prepared by carefully embedding in paraffin, or, better, by embedding in white of egg (see page 12): first moisten with alcohol, and then wash well with water, and soak thoroughly in

the white of egg: coagulate it, and harden in alcohol. Sections are then to be cut of the whole mass, together with the bodies embedded, and they are to be mounted and examined in the usual way.

Attempts should also be made to cultivate the mature spores in a very weak decoction of French plums as above directed for the conidia.

Among the Moulds which appear with constancy on bread kept under a bell-glass, as also on other organic bodies, is *Penicillium*: it may be readily distinguished from *Aspergillus* by its lower growth, more velvet-like appearance, and blue-green colour, while the latter shows a higher growth, so that the individual conidiophores may be seen with the naked eye: its colour is an olive-green.

Remove a small piece from a pure patch of *Penicillium* which has been recognized by the above characters: tease it out with needles, then moisten it with alcohol, and mount in water. Examine it under a high power, and observe the branched, septate mycelium, which frequently forms a very dense mat: this is especially the case if it be grown on Pasteur's solution with sugar. Note that certain branches, which grew up from the substratum, end in a brush of closely arranged parallel branches, and that each branch is terminated by a string of conidia: these are formed by basipetal abstriction, in the same way as in *Aspergillus*.

The conidia may be germinated in the same way as those of *Aspergillus*, and with suitable precautions pure cultures may be grown on various nutritive substrata.

IV. PERONOSPOREÆ

PYTHIUM DE BARYANUM

I. Sow seeds of the common garden Cress (*Lepidium sativum*) thickly in a flower-pot: cover it over with a glass plate, and keep it well watered, so that the young seedlings grow up in an atmosphere saturated with water. After a few days the heads of some of the seedlings may be seen to have bent over, owing to insufficient support of the stem: examination will show that the curvature is a sharp one, so that it is not due to general weakness: further that the stem is thin and flabby at the point of curvature: while fungal filaments may be observed in close contact with the stem at that point, and it is this Fungus (*Pythium de Baryanum*) which is the cause of the disease termed by gardeners "damping off": it is of common occurrence in propagating pits which are kept too warm and moist.

Other members of this, and other allied genera may also be present, but the species above named is almost certain to appear on damp cultures of the common Cress: the difficulty of distinguishing these species from one another may cause apparent discrepancy between the observations and the description given below.

If the Cress cultures be kept damp for some days longer, a thick felt of hyphæ will be formed, which will bind the seedlings together: and finally the disorganization, which usually begins near the base of the hypocotyledonary stem, will spread throughout the seedlings, causing complete rotting.

II. Mount part of a stem of one of the collapsed seedlings in water, and examine under a low power: observe—

1. That the tissues show an abnormal appearance at the point of curvature, their colour is yellowish, and the individual cells show signs of having lost their turgidity.

2. That numerous colourless branched **hyphæ** extend along the surface of the seedling, being most numerous at the point of curvature, and less frequent further up.

III. Tease out a portion of the infected part, as well as of the healthy part above, with needles in water, and mount so that a part at least of the epidermis shall be seen in external surface view; or sections may be cut, the infected part being held between pieces of pith: in such preparations observe—

1. The healthy part of the epidermis with elongated cells, and occasional stomata.

2. The branched, highly refractive, and for the most part non-septate **hyphæ,** running with an irregular, but mostly longitudinal course along the outer surface.

3. Mark especially the points of entry of the Fungus into the host-plant: this may be either—

a. By **perforation** of the outer wall of a cell of the

epidermis; and this is by far the more common; or—

b. By passage of the hypha **through the pore of a stoma**: this is the less common mode.

4. Trace the further course of the hypha through the transparent tissues of the host-plant, noting the **rarity, or complete absence of septa.**

IV. From diseased specimens hardened in alcohol, cut transverse sections: mount in weak glycerine, together with a drop of iodine solution, and examine under a high power. In these specimens the above observations are to be severally confirmed: it is further to be noted that the hyphal filaments traverse the cell-walls of the host, showing a slight constriction at the point of perforation: also that they traverse the whole epidermis and cortex, either passing directly through the cells, or running along the intercellular spaces.

V. Place an infected seedling in fresh water, in a flat watch-glass, and examine it at intervals for a day or two under a low power. Many of the filaments will be seen to form swellings at certain points, which assume a spherical form, are filled with granular protoplasm, and are divided off by a septum from the parent filament, while the thin outer wall assumes a darker colour: these swollen bodies are the asexual reproductive organs, or **resting conidia.** Two types are to be distinguished—

1. **Terminal** conidia, at the ends of the filaments.

2. **Interstitial** conidia, which may appear at any other point on the filament.

It is characteristic of this species that the hypha

should be partially or completely emptied of protoplasm for a short distance below the conidium.

These conidia are capable of withstanding drought, or a temperature below freezing, without losing their vitality.

VI. From a culture containing numerous conidia separate a small portion, and expose it in a watch-glass to a relatively considerable bulk of fresh water: examine the culture at intervals under a low power. Some of the conidia will be seen to germinate by the formation of tubular hyphæ similar to those which produced them.

De Bary and Hesse have also described how certain of these swellings, differing in no structural characters from the directly germinating conidia, develop as **zoosporangia,** by the formation of a lateral beak-like outgrowth, into which the protoplasmic contents pass: a division of the protoplasm then takes place, to form numerous **zoospores,** which escape, and after a motile period, settle and germinate as new individuals. This may often be observed in mixed cultures on placing the specimen in a considerable bulk of fresh water.

VII. Continue at intervals the observation of those cultures which have already produced conidia: the formation of the **sexual organs** will frequently be seen to succeed that of the conidia.

a. The **oogonium** resembles at first the conidium in being spherical, and about of equal size with it, and is partitioned off by a septum; a central spherical mass of protoplasm (the **ovum**) is to be recognized.

b. The **antheridium** arises as a branch, either from the same filament as the oogonium, or from another: its apex is cut off by a septum, and it comes in

close contact with the oogonium: a cylindrical process from it passes through the wall of the oogonium, and gains access to the ovum.

c. In more mature specimens the oogonium contains a single round, distinctly walled cell (the **oospore**), which lies freely within it.

In order to follow the process of fertilization, which is to be seen with distinctness in this plant, portions of a well-nourished culture should be cultivated in a damp chamber, in a suspended drop of water: by selecting an oogonium and antheridium of suitable age, the actual transfer of the granular protoplasm from the antheridium to the ovum may be followed. (See Marshall Ward, Q.J.M.S. xxiii., page 490, &c.)

Cystopus candidus (White Rust of Shepherd's Purse)

Observations on *Cystopus* are so easily made that they should not be omitted. The Fungus is commonly to be found growing parasitically upon *Capsella Bursa-pastoris*, and others of the *Cruciferæ*, causing in summer white eruptions, accompanied by swelling, and greater or less malformation of the vegetative organs, and even of the flower and fruit. It must not be confounded with the equally common *Peronospora parasitica*, which is to be found at similar times on similar plants, and bears a superficial resemblance to it.

Note with a lens the white patches: some will be seen to be still completely covered by the epidermis, in other cases rupture will have taken place, and the mealy mass of white **conidia** is thus exposed.

Cut longitudinal sections of the stem of *Capsella* so as to traverse one of the blotches, and, examining under a high power observe—

1. The general arrangement of tissues as in a typical Dicotyledon.

2. The colourless non-septate **hyphal filaments,** which traverse the intercellular spaces, not only of the cortical parenchyma, but also of the pith : note carefully the button-like **haustoria,** which the Fungus puts out through the cell-walls into the cavities of the cells of the host.

3. Where the section has traversed the white blotch, the epidermis is separated from the subjacent tissue by a mass of hyphal filaments, each of which terminates in a chain of **conidia** formed successively by abstriction.

4. Frequently, and especially in material taken in autumn, the sexual organs of the Fungus are to be seen borne on hyphal filaments within the tissue of the host. The main characters of the **antheridium** and **oogonium** correspond to those of *Pythium :* when ripe the **ovum** is covered by a dark-coloured and rugose **exospore,** as well as by the colourless inner layers (**epispore** and **endosporc**).

Attempts should be made, by placing fresh conidia in a drop of water on a slide, to observe their germination : the contents divide into a number of parts, which escape as motile **zoospores.** Further, the behaviour of these during germination on a fresh leaf of *Capsella*, and the entry of the germinal tubes through the pores of the stomata are to be observed.

Oospores are also to be dissected out from the tissues of the host plant in spring (*i.e.* after the period of rest), and to be cultivated in fresh water: their germination is to be observed, the protoplasm dividing into a large number of swarm-spores, which escape and develop further in the same way as the zoogonidia.

Observations may also be made on the Potato Fungus (*Phytophthora infestans*) the mycelium of which permeates the tissues of the Potato plant, while its branched conidiophores project through the stomata.

V. MUCORINEÆ

MUCOR MUCEDO, *Fres*

I. If a slice of bread be soaked in water, and kept under a bell-glass, various moulds will make their appearance upon it: about the fourth or fifth day there will be seen a mould, which at first appears white and flocculent, producing long unbranched stalks, which terminate in round heads, white at first, and subsequently becoming black: this will be *Mucor Mucedo.* It may also be obtained on horse-dung kept under a bell-glass, and on various other substrata.

II. Remove a very small piece of the bread bearing the mould, and tease it out gently in water: mount and examine under a low power: note—

1. Relatively thick, non-septate hyphæ, which ramify in the substance of the bread.

2. Relatively thin branches, which are produced from the thick ones, and themselves, branching repeatedly, produce a very extensive system of minute fibrils.

3. Hyphæ similar to (1), which however grow erect in the air (**gonidiophores**), each bearing at its summit one spherical **sporangium**: this will certainly have

been damaged in the process of preparation. For the observations on its structure see below.

By hardening in alcohol, or by fixing with picric acid, and subsequently treating with staining reagents, it may be demonstrated that numerous nuclei are present in the hyphæ of *Mucor*.

It has been stated above that the hyphæ of *Mucor* are not septate: this is the case in young cultures, but preparations should also be made from old cultures, after the production of the sporangia: in these numerous septa may be found.

It has also been stated that the gonidiophores are unbranched: that is so in the large majority of cases, but in old cultures lateral branches may be found arising below the sporangium, and themselves terminated in turn by a sporangium.

III. Cut off a number of mature sporangia with scissors from the flocculent growth, treating them very gently, so as to avoid damage: mount them in alcohol, and examine them quickly under a low power: observe—

1. The cylindrical **gonidiophores,** terminated by—

2. The spherical and dark-coloured **sporangium,** with its dense contents, and its very thin limiting wall, often bearing small radiating projections.

3. Towards the point of attachment to the stalk a clearer space may be recognized in the contents; this indicates the position of the **columella.**

Add a drop of water, and draw it under the cover-slip with blotting-paper, watching the effect upon the sporangia: as the water gains access to the sporangia, they burst suddenly, and the wall may be torn to fragments so minute that it cannot be recognized again. Meanwhile the contents, the swelling of which caused

the rupture, gradually distend, and may be recognized as consisting of—

4. Numerous oval **spores,** with smooth walls.

5. An intermediate **mucilaginous substance** which is capable of swelling, and thus effects not only the bursting of the sporangium, but also the dispersal of the spores.

6. After the swelling and dispersal of the spores are complete, there will be seen remaining a spheroidal body (the **columella**), which is the distended septum of separation of the sporangium from the gonidiophore: round its base may often be traced the remains of the wall of the sporangium as a ragged fringe.

The minute projecting bodies on the surface of the sporangium are not of constant occurrence, though often present: they consist of oxalate of lime.

According to the conditions of nutrition of the Fungus, there may be very considerable variety in the size of the sporangia, and in the number of the spores produced: under peculiar circumstances the sporangia may be of so reduced a form that the columella is absent, and the number of spores may be less than ten.

As a second type *Pilobolus crystallinus* may be taken: this Fungus appears with great constancy on dung of the horse or cow, if kept under a bell-glass for about ten days: it is specially interesting because of the mechanism for shooting off the sporangium bodily from the gonidiophore. The sporangia may thus be commonly found adhering to the inner surface of the bell-glass which covered the culture.

IV. With similar precautions to those taken in the case of the spores of *Eurotium Aspergillus* (p. 486), sow spores of *Mucor* in a drop of a sterilized decoction of horse-dung, or of French plums, or other suitable

solution: the swelling and germination of the spores and the formation of the branched, non-septate mycelium are to be watched; and drawings may with advantage be made at intervals, so as to record the progress of the cultures.

The *Torula* condition may be induced in certain of the species of *Mucor*, especially in *M. racemosus* (though not so readily in *M. mucedo*), by growing the mycelium immersed in a nutritive solution such as Pasteur's solution: the hyphæ then become partitioned off by transverse septa into short **gemmæ.** During this stage alcoholic fermentation may be effected by it. On being re-exposed to the air, under other suitable conditions the gemmæ may germinate and produce a normal mycelium. Observations should be made on these points.

SPORODINIA GRANDIS, *Link*

V. *Mucor mucedo* also reproduces itself by means of **zygospores,** which are of such size that they may be detected with the naked eye as black bodies which project slightly from the substratum; but they are not of constant occurrence, and may frequently be looked for in vain. Accordingly it will be found more convenient and successful to study the development and structure of the zygospores in an allied form, in which they are produced in profusion, viz. in *Sporodinia grandis*, Link. (= *Syzygites megalocarpus*, Ehr.).

Sporodinia is a fungus which may frequently be found in autumn, growing parasitically on many of the larger, fleshy Hymenomycetes, especially on *Russula*, or *Boletus:* it appears as a greyish or brown flocculent growth, and the zygospores are of such a size that they can readily be seen as reddish-brown bodies with the

naked eye. While a part of the mycelium ramifies in the tissue of the host, the zygospores are borne on aërial branches: they may thus be easily recognized as brown bodies, visible to the naked eye.

Tease out a small piece of the flocculent mycelium gently in water: examine under a low power, and observe—

1. The branched **hyphæ,** which are light-coloured, and rarely septate when young, but assume a brown colour, and form numerous transverse septa at irregular intervals as they grow old.

2. The large brown **zygospores,** each supported by two thicker, club-shaped hyphæ (*Syzygites* form).

3. The relatively small **sporangia** borne on branched gonidiophores, and having a structure similar to those of *Mucor* (*Sporodinia* form).

Compare a number of zygospores in various stages of development, and observe in them the following points—

1. The swelling of two neighbouring mycelial filaments (**suspensors**), and their assumption of a position with their two swollen ends opposite one another.

2. The formation of transverse **septa** cutting off the apical part of each suspensor, thus forming the two **gametes.**

3. The two **gametes** in close contact with one another, while the walls at the point of contact are gradually absorbed, the absorption beginning at the central point: the two protoplasmic bodies thus coalesce to form the **zygote** or **zygospore.**

4. The increase in size of the zygospore, its contents becoming dense and oily, while the wall at the period

of maturity consists of the following successive layers—

a. The **primary membrane** of the gametes, which remains thin, but persistent as an external covering.

b. The **epispore,** which is a dark-coloured firm or brittle layer with hemispherical wart-like outgrowths from the surface.

c. The **endospore,** which is thicker and more transparent.

Note how numerous though irregular are the septa in mycelium which has produced zygospores.

It is not an uncommon thing in *Sporodinia* to find that the two gametes may not come in contact, and no zygote be found; but still each gamete may develop into a body resembling a zygospore in the character of the wall, the contents, and in the mode of germination. These bodies are called **azygospores.**

Attempts should be made to germinate the zygospores. This may be done by keeping them under observation in fresh water for some weeks during the autumn, changing the water frequently: the brown epispore ruptures, the endospore protrudes, and forms two to four germinal filaments. If such germinating spores be now cultivated on a moist substratum, the filaments may form gonidiophores of the *Sporodinia* type.

APPENDIX A

THE following list of reagents is not intended to be an exhaustive catalogue of the various substances in use in the Botanical Laboratory: it includes, however, those reagents which are considered to be of the greatest importance, together with notes on their proper preparation, and uses.

Acetate of Potash. A strong solution in water is used as a mounting medium for preparations of green parts of plants: in this solution they retain their green colour for a long time. Aluminium acetate may also be used for the same purpose (p. 53).

Acetic Acid. This is usually used as a dilute solution in water (1 per cent.): it dissolves calcium carbonate with evolution of bubbles of CO_2 (see p. 140): it brings out the nuclei very clearly, and with this object in view it is used with methyl-green: it may also be employed as a corrective after treatment of a preparation with potash, if the tissues have become too transparent. Glacial acetic acid is also sometimes used in the preparation of the apex of *Fucus*.

Alcohol is of universal use as a solvent, precipitant, and hardening agent. Absolute alcohol is the best, but for most ordinary work strong methylated spirit will do (see p. 5, &c.). It dissolves chlorophyll and other colouring-matters (pp. 47, 128), resins (p. 65), ethereal oils, and some fixed oils (p. 223): wax is soluble in hot alcohol. It precipitates some substances, such as sugars (p. 222), inulin (p. 119), and asparagin (p. 228). It coagulates proteids, and has a peculiar action on some crystalloids. It acts as a hardening agent on cell-walls, sometimes rendering them too brittle: this may be overcome by soaking the material, before

cutting sections, in a mixture of equal parts of alcohol and glycerine.

Alkanna (the root of *Anchusa tinctoria*) is used as a test for resin (p. 65), caoutchouc and oils (p. 223). The alcoholic solution of alkannin, as supplied by the dealers, may be used for this purpose; but it is found better to use sections of the dry alkanna root, as described on p. 66. The substance alkannin is not readily soluble in alcohol: the best way to prepare it is to make a saturated solution in absolute alcohol, and then dilute with strong methylated spirit: it is, however, obvious that, as resins are soluble in alcohol, the globules of resin which it is desired to stain are liable to be dissolved on application of the reagent.

Ammonia. The solution in water is often used for clearing preparations instead of potash, as its action is less intense. It is used with nitric acid as a test for proteids, and with copper sulphate as a solvent for some forms of cellulose (see below, Copper Sulphate).

Ammonium Molybdate, used, dissolved in a strong solution of ammonium chloride, as a reagent for the detection of tannin, with which it gives a voluminous yellow precipitate.

Aniline Sulphate and **Chloride** are used as reagents for lignified cell-walls, which they stain yellow, while no other parts of the tissue are coloured by them. A saturated solution of either of these substances is made in distilled water, filtered, and a few drops respectively of sulphuric or hydrochloric acid are added, so that the solution shall give a distinctly acid reaction: or a solution may be made in alcohol, and then be diluted with water.

Aniline Violet (Hanstein's). This is prepared by dissolving equal parts of fuchsin and methyl-violet in alcohol. It stains cellulose cell-walls of a faint violet colour, and lignified cell-walls deep violet. It is especially useful for bringing out the different parts of the bast, since the bast-fibres stain red, whereas the sieve-tubes and the parenchyma scarcely stain at all. The protoplasm is stained pink: amyloid substances, gums, and nuclei stain different shades of red, resins blue, and tannin brick-red.

Asparagin. A saturated solution of this substance in water is used as a test for asparagin which has been already precipitated

by alcohol. It may be prepared from the substance as supplied by the dealers, or by extraction of seedlings of *Lupinus luteus* with water, and evaporation (see p. 228).

Asphalte is used for sealing up slides in which glycerine has been used as a mounting medium (see p. 52): it is liable to become very brittle after a time, and to prevent the cement breaking away, it may with advantage be covered with a layer of gold-size. It may be obtained ready for use from the dealers.

Benzol, used as a solvent for various substances, *e.g.* the coagulum of latex, ceric acid (p. 40), &c.

Brunswick Black may be bought ready prepared from dealers in microscopic requisites: it is used for sealing up slides (p. 52).

Calcium Chloride has been recommended for the preparation of sections of growing points: it is to be used as directed on p. 50.

Callus-reagent of Russow is prepared by mixing equal volumes of chlor-zinc-iodine, and of the solution of iodine in potassium iodide: it stains the callus of sieve-tubes a deep brown (p. 115).

Canada Balsam is to be used dissolved in benzol and in such quantity that it shall have the consistency of a syrup. It is used as a mounting medium for sections previously treated with alcohol, and then, with either oil of cloves, turpentine and creosote, or cajeput oil (see p. 52); it is also used for sealing up slides.

Cane-Sugar. The concentrated solution in water is sometimes used, together with strong sulphuric acid, as a test for proteids. A dilute solution (1 per cent., or more) is useful for mounting living cells for observation under the microscope (see p. 202).

Carbolic Acid (see Phenol).

Carmine. The two best preparations of carmine are those of Beale and Thiersch.

1. Beale's Carmine.—To prepare this, 0·6 gramme of carmine is dissolved in 2 c.c. of boiling solution of ammonia; the solution must then stand for an hour or so to cool, and to allow of the escape of the superfluous ammonia; to the solution are added 60 c.c. of distilled water, 60 grammes of glycerine, and 15 grammes of absolute alcohol. The mixture must be allowed to stand for some time; it is then to be filtered.

2. Thiersch's Carmine.—4 grammes of borax are dissolved in

56 c.c. of distilled water ; to this 1 gramme of carmine is added, and then twice its volume of absolute alcohol is added to the liquid. After filtration the liquid is ready for use.

Carmine has but little differentiating power : it readily stains the protoplasm and the nucleus ; Thiersch's preparation is especially useful for bringing out the structure of the nucleus. It can very well be used for sections which have been previously treated with picric, chromic, and osmic acids. The time during which the section is to be exposed to its action varies very much ; the rule is that the most satisfactory results are obtained by a prolonged immersion in a dilute solution. In case of over-staining, the section may be washed for a moment in water to which a trace of ammonia has been added.

Preparations stained with carmine are best mounted in glycerine. (See also Picro-carmine.)

Chloral Hydrate is used, together with iodine, for the detection of starch-grains included in the chlorophyll-corpuscles. Dissolve 8 parts chloral hydrate in 5 parts of water, and add crystals of iodine, which will dissolve slowly and colour the solution. The material to be tested should be bleached with alcohol, and then be laid in the solution for twelve to twenty-four hours (see pp. 46, 130).

Chloroform is used as a solvent for various substances, *e.g.* oils, coagulum of latex, &c.

Chlor-Zinc-Iodine (Schulze's Solution) is the best differentiating reagent, and the one most generally used, but the chief objection to it is that, as in the case of other preparations of iodine, the stain in not permanent. There are various ways of preparing it, but the best is as follows :—

1. Dissolve 110 grammes of zinc in 300 c.c. of pure hydrochloric acid, and evaporate to 150 c.c. (sp. gr. 1·8).

2. Dissolve 12 grains of potassium iodide in as little water as possible, and add 0·15 grammes of crystals of iodine.

3. Mix (1) and (2).

This reagent may however be obtained ready prepared from dealers in microscopic requisites. It may be used either for fresh material, or after treatment with picric acid, or alcohol : the colouring of cellulose walls is intensified if the objects have been

previously treated with potash, and the alkali thoroughly washed out.

Under this reagent cellulose walls turn blue, or violet (p. 37), lignified walls yellow, or various shades to a sherry brown (pp. 39, 95), corky walls yellow or brown (p. 39), protoplasm brown, while starch-grains swell and turn blue (p. 45).

Chromic Acid. A strong aqueous solution of this acid, 10 per cent., dissolves lignified and cellulose cell-walls: cuticularized cell-walls resist its action; but they become very transparent, and may be easily overlooked. A dilute solution brings out the stratification of cell-walls very clearly. A 1 per cent. solution may be used in the preparation of Seaweeds.

Clove-oil is used as a clearing agent before mounting specimens which have been treated with alcohol in Canada balsam (p. 52).

Copper Sulphate is used in the preparation of Fehling's fluid (see below), and the preparation of ammoniacal solution of cupric hydrate (see below).

Corallin (Rosolic Acid). A solution of corallin in a 30 per cent. solution of sodium carbonate colours lignified tissue, the callus of sieve-tubes (p. 115), and starch-grains pink.

Creosote is used together with turpentine as a clearing agent before mounting in Canada balsam (p. 52): 1 part of creosote and 4 parts of turpentine are to be shaken well together, and set aside till the cloudiness formed on their first mixing disappears.

Cupric Hydrate. The ammoniacal solution of cupric hydrate is used as a solvent for pure cellulose (p. 37). To a solution of copper sulphate in water add dilute potash: collect the precipitate on a filter, wash with water, and then dissolve it in a little strong ammonia: this solution, which is of a dark blue colour, must be prepared fresh each time it is required for use.

Dammar, dissolved in warm turpentine, and evaporated to the consistency of syrup, is sometimes used as a mounting medium instead of Canada balsam: it does not set so firmly as balsam, and it is well to seal up slides in which it has been used (p. 52).

Ether is used as a solvent for wax, oils (p. 223), &c. When very small objects have been embedded in paraffin or cocoa-butter, it may be convenient to dissolve off the fragments of embedding

material with ether: the small sections will then be readily found, and collected.

"**Eau de Javelle**" is recommended as a clearing agent for growing points, and other merismatic tissues: the cell-contents swell under its action, and the cell-walls which remain may then be easily seen. It is prepared by adding to 2 pints of water 2 ounces of chloride of lime, and 4 ounces of carbonate of potash or of soda. Objects treated with it are to be washed with water, then with dilute acetic acid, and should be mounted in glycerine.

Eosin is used in strong solution in alcohol, or in water, for demonstrating the structure of sieve-tubes (pp. 113, 116).

Fehling's Fluid is used as a test for grape-sugar (p. 222): the following directions for its preparation are given in Foster's *Practical Physiology*:—

a. Dissolve 34·65 grammes of pure crystallized cupric sulphate in about 160 c.c. of distilled water.

b. Dissolve also 173 grammes of pure crystallized potassic-sodic tartrate in 600 to 700 grammes of sodic hydrate (sp. gr. 1·12).

Add (*a*) to (*b*), stirring well to cause a thorough mixture, and dilute with distilled water to a litre.

Fehling's fluid should be fresh made whenever it is required, since it decomposes on keeping; it will keep some little time if kept in a cool place in the dark, and in completely filled, well-closed bottles (Hoppe-Seyler).

The solution (*b*) may be prepared, and kept for adding to (*a*) freshly prepared when required.

Before using a kept solution to test for sugar, always boil a little of it by itself to see if any reduction will take place.

From 1 c.c. of this solution the copper is completely reduced by 0·005 grammes of grape-sugar.

Ferrous Sulphate is used in dilute solution in water, to which a drop of nitric acid has been added, as a test for tannin.

Fuchsin is used in solution in alcohol, for bringing out the structure of thickened cell-walls, and especially the outer walls of the epidermis (p. 246), and corky walls: the sections should have been previously treated with alcohol. When a section has been stained with fuchsin, and washed in absolute alcohol, the

coloration is removed from all parts excepting the corky and cuticularized walls.

Glycerine is the most generally used medium for mounting, as it has the advantages of a high refractive index, and of not being subject to evaporation. It may be applied either pure or diluted: pure glycerine is to be used, after hardening in alcohol, when it is desired to observe the details of the protoplasm, *e.g.* in the preparation of the contents of the embryo-sac (p. 204); dilute glycerine (1 part glycerine, 1 part water), is however, of most general use.

Glycerine Jelly is a suitable mounting medium for many objects (p. 52): it may be bought ready for use from dealers in microscopic requisites; or it may be prepared according to Kaiser's receipt, as follows:—1 part by weight of finest French gelatin is to be soaked for about two hours in 6 parts of distilled water: 7 parts of chemically pure glycerine are added, and to about 100 grammes of this mixture 1 gramme of carbolic acid is added. The whole mixture is to be warmed and continually stirred for 10–15 minutes, till the fluid is clear, and then to be filtered through glass-wool.

Gold Chloride is sometimes used in a 1·0 per cent. or 0·5 per cent. solution in water as a delicate stain for protoplasm.

Gold-Size is to be obtained from dealers in microscopic requisites: it is used for sealing up slides, and a layer of it may with advantage be applied after sealing with asphalte, or Brunswick black.

Gum Arabic is occasionally used as an embedding medium for very small objects (p. 12).

Hæmatoxylin. A number of preparations of this colouring-matter are in use; of these the following are those generally employed for vegetable tissues:—

1. Alum Solution of Hæmatoxylin.—Dissolve 0·35 gramme of hæmatoxylin in 10 c.c. of water, and add to it a few drops of a solution of alum consisting of 1 gramme of alum to 10 c.c. of water.

2. Kleinenberg's Hæmatoxylin.—Saturate some 70 per. cent. alcohol with calcium chloride; let the mixture stand for twelve to twenty-four hours over alum, shaking occasionally; add 8

parts of 70 per cent. alcohol ; filter, and then add a solution of hæmatoxylin in absolute alcohol until the liquid has a purple-blue colour ; let it stand in a corked bottle exposed to sunlight for about a month ; it is then fit for use. The liquid is to be diluted as required with alum solution. This preparation is most generally employed, and it may be bought from the dealers ready for use.

3. Expose a few crystals of hæmatoxylin to the action of gaseous ammonia in a watch-glass under a bell-jar : then add water, and a good colouring fluid is obtained. The disadvantage of this is that it has to be freshly prepared every time it is required.

The alum solutions will stain all parts of the cell, including the cell-wall. Their especial uses are (*a*) to make the cell-walls more evident when they are naturally transparent and colourless ; (*b*) to stain the protoplasm, so as to make its intimate structure apparent ; (*c*) to stain the nucleus, so as to demonstrate its presence and to show up its structure.

The ammoniacal solution is especially adapted for differentiated staining. If a dilute solution be used, the first thing to become stained is the chromatin of the nucleus, then, after a time, the rest of the nucleus (achromatin), then the protoplasm. The cell-walls do not stain with this fluid, or only slightly. Kleinenberg's hæmatoxylin stains in a few minutes, whereas the alum solution is much slower in its action.

Hæmatoxylin may be used either for fresh material, or for sections which have been previously hardened with alcohol, or with picric or chromic acid. In the latter case the sections must be washed repeatedly in distilled water to remove every trace of the acid, which, if present, would interfere with the proper action of the hæmatoxylin. If the section becomes too deeply stained, as sometimes happens when the alum-hæmatoxylin is used, the excess of colouring-matter may be removed by washing with a solution of alum in water.

Sections stained with alum, or with Kleinenberg's hæmatoxylin, are to be mounted in Canada balsam, or Dammar ; those stained with the ammoniacal solution are to be mounted in glycerine.

Hoffmann's Blue. Used in solution in dilute alcohol slightly acidified with acetic acid : it is a useful reagent, inasmuch as it

stains the protoplasmic cell-contents and not the cell-wall: it stains also the callus which closes the perforations of the sieve-plates during the winter in perennial plants. It is also used, together with sulphuric acid, for demonstrating the continuity of protoplasm through cell-walls: in order to do this a small quantity of the dry substance is dissolved in strong sulphuric acid in a watch-glass: sections, preferably of fresh material, are then immersed in it for a short time, then washed with water, and mounted in glycerine (see p. 214).

Hydrochloric Acid. Used, in very small quantity so as to give an acid reaction, with aniline chloride, phloroglucin, or carbolic acid, as a test for lignin. By itself the acid turns lignified cell-walls yellow; when its action is prolonged, the cell-walls become violet, owing to the presence of various substances such as phloroglucin, coniferin, and pyrocatechin.

Iodine is one of the most useful reagents: it is prepared for use in various ways. The most important are the following:—

i. Make a strong solution of potassium iodide in distilled water, add to this crystals of iodine and set it aside for some hours, shaking it occasionally: dilute this solution with distilled water to the colour of brown sherry. The reagent may also be prepared by diluting the *liquor iodi* of the Pharmacopœia. This is the ordinary iodine solution in common use in the laboratory.

ii. The alcoholic solution may be prepared by dissolving crystals of iodine in alcohol, and diluting with alcohol to a dark sherry colour; also by diluting the *tinctura iodi* of the Pharmacopœia: in the absence of water this solution does not give the blue reaction with starch (p. 46).

iii. A solution of potassium iodide and iodine in pure glycerine is sometimes used in the treatment of crystalloids (p. 225).

iv. The solution of iodine in chloral hydrate is used for detection of included starch-grains (see above, Chloral Hydrate).

v. For the solution in chloride of zinc (Schulze's solution), see above, Chlor-Zinc-Iodine.

The ordinary solution of iodine (i.) stains proteid substances, and especially the nucleus, brown (p. 27); cellulose faintly yellow; cuticularized and lignified walls yellow (p. 39); gum purple and starch blue (p. 45). Together with sulphuric acid, iodine colours

cellulose blue (p. 36), a reaction similar to that with chlor-zinc-iodine (p. 37).

Methylene Blue is used in solution in water: it stains the cell-wall, but not the protoplasm.

Methyl-green. A tolerably strong alcoholic solution of this is used. The sections of the object, which must have been previously kept in absolute alcohol, are to be treated with the staining-fluid for from 5–25 minutes, then quickly washed with distilled water, and mounted in glycerine. The nucleus stains of a green or bluish-green colour, the protoplasm remaining uncoloured. It is especially good for staining nuclei which are dividing, and for bringing out the nuclei in the cells of Fungi, and of the Siphoneæ, for which purpose Strasburger recommends the following method:—The fresh object or section is mounted in 2 per cent. acetic acid, to which a little methyl-green has been previously added: the nuclei are fixed almost instantaneously and at the same time stained. These preparations may then be washed in 1 per cent. acetic acid, and be mounted in weak glycerine and acetic acid. Objects stained with methyl-green fade very rapidly.

Methyl-violet. This is used in concentrated alcoholic solution. It is especially useful for staining Bacteria. A few drops of the solution are added to 15–20 c.c. of distilled water, and a drop or two of the mixture should then be placed on the Bacteria-membrane (zooglœa), and be allowed to remain there for a short time until the membrane appears to be coloured: if the solution used be too strong, the substance between the Bacteria will become stained. The colouring matter is then washed off with distilled water, or better with a 10 per cent. solution of acetate of potash. The preparation may then either be allowed to dry in the air and be then mounted in Canada balsam, or it may be mounted in a 50 per cent. solution of potassium acetate in water.

A useful preparation of methyl-violet is the following:—Some of that substance is dissolved in strong sulphuric acid, forming a brownish-green solution: on the gradual addition of water the violet colour reappears. This is especially useful for sieve tubes. If a section be treated with this fluid for a

short time, and be then washed with water, it will be found that the cell-walls have become swollen and transparent, that the protoplasm has become deeply stained, and that the sieve-plates are very well brought out. Lignified tissues treated with this fluid assume a yellow colour, as they do when treated with aniline sulphate.

Moist Chamber (see Water).

Nitric Acid colours cuticularized cell-walls and proteids yellow; it also causes swelling up of cellulose and of lignified cell-walls. When diluted with water it is useful for dissolving the crystals of calcium oxalate which are frequently present in the cells (pp. 109, 178). It is used with ammonia as a test for proteids (xanthoproteic reaction); with potassium chlorate as a test for suberin, and as Schulze's macerating fluid (p. 104).

Olive Oil is used as a medium for mounting aleurone-grains, so as to see them unaltered (p. 223).

Orcin. A solution in alcohol is used as a test for inulin. Sections are to be soaked in the solution and subsequently warmed with strong hydrochloric acid: an orange-red colour shows the presence of inulin.

Osmic Acid is used in 0·1–1·0 per cent. solution in water, for fixing and hardening protoplasm (p. 5): it also stains fats black (p. 223). The solution should be kept in a well-stoppered bottle in the dark.

Paraffin is used as an embedding medium for small or delicate objects (p. 10). Paraffins of varying hardness and temperature of melting-point may be obtained: the best for ordinary use is a mixture which shall melt at a temperature of 50° to 60° C.

Phenol (Carbolic Acid). Used, together with hydrochloric acid, as a test for lignin. The best preparation of it is its solution in hydrochloric acid: this is prepared by dissolving carbolic acid in warm hydrochloric acid, adding, whilst the mixture is cooling, sufficient hydrochloric acid to dissolve any precipitate that may be formed. Lignified cells, treated with this mixture and exposed to sunlight, assume a bright green colour in consequence of the presence of coniferin. It may also be used, instead of creosote, together with turpentine, as a clearing

agent, before mounting in Canada balsam. A small quantity is to be added to glycerine jelly to prevent the growth of Fungi.

Phloroglucin. Dissolve some phloroglucin in methylated spirit, and gradually add strong hydrochloric acid till precipitation begins; the liquid is then ready for use: in sections treated with it lignified walls assume a bright red colour.

Picric Acid. A saturated solution in water is very generally used for fixing the protoplasm of the cell as nearly as possible in the form which it held during life (p. 5). It is, however, objectionable, owing to the difficulty in completely washing it out from the specimens before hardening in alcohol, and in most cases treatment at once with absolute alcohol is to be preferred. In some cases, such as delicate Algæ, it is well to dilute the saturated solution with an equal volume of water.

Picro-carmine (or ammoniun picro-carminate) is prepared by adding a strong ammoniacal solution of carmine to a quantity of concentrated solution of picric acid in water, until a precipitate begins to be formed; it is then evaporated to about one-fifth of its bulk, filtered, and the filtrate is evaporated to dryness. The crystalline residue is dissolved in water so as to make a 5 per cent. solution, and this may be diluted as occasion requires.

Another method (Gage) is to dissolve a quantity of picric acid in 100 parts of water, and an equal quantity of carmine in 50 parts of solution of ammonia; these are then mixed, filtered, evaporated to dryness, and the residue dissolved in 100 parts of water.

Picro-carmine is used especially for staining nuclei, the staining being more uniform than when carmine alone is used: it has this further advantage, that a prolonged exposure to it does not produce overstaining, as is the case with the other preparations of carmine. The objects should be previously kept for some time in absolute alcohol. If it be desired to retain the double staining which this reagent produces, the sections must be mounted at once in glycerine; but if the carmine staining only is required, the sections must be washed in water, which will dissolve out the picric acid. When stained sections are mounted in glycerine,

a small quantity of picro-carmine must be added to the glycerine in order to preserve the colours.

The various preparations of carmine can be used as well for tissues which have been hardened in chromic, picric, or osmic acid, as for fresh tissues, but the former stain less readily.

Picro-nigrosin: make a saturated solution of picric acid, add crystals of nigrosin, and allow them to dissolve: steep the specimen in it, and allow time for slow staining; this reagent may be used for simultaneous fixing and staining of delicate tissues, and is especially recommended in the preparation of *Spirogyra* and other Algæ, and for Fungi.

Potash may either be used in a dilute solution (1–5 per cent.), or in a strong solution in water. A dilute solution is commonly used as a clearing agent (p. 49): it causes cell-walls and starch-grains to swell, especially when heated, and it dissolves sphere-crystals of inulin, crystalloids, and most aleurone-grains, and saponifies fats. It gives a reddish colour to cells in which tannin is present.

A strong solution may be used as a test for suberin: when sections of cork are boiled in strong potash, the suberin escapes in the form of yellow viscid drops; when the sections are only slightly warmed in the solution, the cuticularized walls assume a yellow colour (p. 39).

A concentrated solution of caustic potash in alcohol is sometimes used with good effect in the preparation of apical meristems, but specimens so treated cannot be permanently kept.

Potassium Acetate (see Acetate).

Potassium Bichromate is used in dilute solution in water as a test for tannin, which it colours dark brown: the 1 per cent. solution in water may also be used for hardening tissues.

Potassium Chlorate is used together with nitric acid as a macerating agent, and as a test for suberin (see below, Schulze's Macerating Fluid).

Russow's Callus-Reagent (see above, Callus-reagent).

Safranin. This may be used in solution in absolute alcohol. It is especially adapted for staining sections which have been previously hardened with chromic or picric acid; it is not so good for those which have been treated with osmic acid. The

sections must be well washed in distilled water, and then placed in a small quantity (1 c.c.) of the saturated alcoholic solution mixed with an equal volume of distilled water; they require to be left for several hours in the staining fluid. They must then be removed, and washed for a short time in alcohol; then they must be placed in absolute alcohol, and kept there until they appear transparent. The sections can now be mounted in distilled water in order to see if the results are satisfactory, or, if they are to be preserved, they must be cleared with oil of cloves, and mounted in Canada balsam or Dammar.

By this means very successful preparations of the structure of nuclei can be obtained.

Schulze's Macerating Fluid. One gramme of potassium chlorate is dissolved in 50 c.c. of nitric acid; or crystals of potassium chlorate may be left to dissolve to saturation in a small bottle of nitric acid. This reagent is to be used only in small quantities, and the process of maceration should not be conducted in near proximity to microscopes, or other metallic apparatus.

It is used as a macerating fluid for separating the constituents of woody tissues from one another, this result being obtained by the solution of the middle lamella. The tissue to be macerated is cut into small chips, and boiled in the fluid for a short time in a test-tube; the fluid is then poured off and the residue collected on a filter, and well washed with water: the specimens may then be mounted in glycerine (see p. 104).

Schulze's Solution (see above, Chlor-Zinc-Iodine).

Sodium Chloride is used as a 10 per cent. solution, or as a saturated solution in water, as a solvent for proteid-crystalloids (see Appendix B).

A more dilute solution (1–5 per cent.) is used for inducing plasmolysis (see pp. 26, 31, &c.).

Sulphuric Acid. This is used either concentrated, or dilute (1 to 3 of water). It causes, in either case, the swelling up of cellulose cell-walls, starch-grains, &c. (p. 46); when cellulose cell-walls which have been previously saturated with solution of iodine are treated with sulphuric acid, they turn blue (p. 36).

Concentrated sulphuric acid dissolves cellulose and starch, but cuticularized or corky cell-walls and the middle lamella of ligni-

fied cells resist its action (see p. 39). It is used with cane-sugar, as a test for proteids, and a few drops of it are added to a solution of aniline sulphate as a test for lignin.

It may also be used as a solvent for crystals of calcium oxalate (p. 178).

Turpentine is used with creosote, or carbolic acid, as a clearing agent before mounting in Canada balsam (p. 52).

Water may be used as a mounting medium (p. 24), and as a solvent for various reagents ; it may also be used for the cultivation of small organisms, or pollen-grains, spores, or Fungi, under the microscope, and for this purpose a **moist chamber** is to be constructed as follows :—

A piece of thick rough cardboard is cut to the size of the glass slide, and a circular hole is punched out of the middle of it of such a size as to be completely covered by a cover-slip. The piece of cardboard is then soaked in water (or boiled in water when pure cultures of Fungi are to be made), so as to saturate it, and placed on the glass slide. A drop of water (or solution as described below) is placed on the cover-slip, the object is immersed in it, and the cover-slip is then inverted over the hole in the piece of cardboard. Thus the object is suspended in a drop of liquid on the under surface of the cover-slip. Any loss from the chamber by evaporation is prevented by occasionally wetting the cardboard on the slide with freshly boiled, distilled water.

The liquid to be used will of course vary with the nature of the object to be observed. In the case of Algæ, water may be used ; in the case of Fungi, decoctions of various organic substances (fruits, animal tissues, &c.), or a solution of sugar, according to the habit of the Fungus. For observing the germination of the spores of Mosses and Ferns, water will suffice ; but in the case of pollen-grains a solution of sugar is necessary (1–20 or even 30 per cent., the concentration being different for different plants) (p. 202) ; for observing the process of cell-division in the hairs on the stamens of Tradescantia, a 2 per cent. sugar-solution may be used (p. 84).

White of Egg is sometimes used as an embedding medium (see p. 12).

APPENDIX B

THIS appendix includes in a tabular form, as being convenient for reference, the more important reactions of the parts of the vegetable cell, and of bodies commonly contained in it: references are given in the case of the more important reactions, to the pages in the text where descriptions are to be found for carrying out the tests on suitable material.

Cellulose Cell-walls.

i. Coloured faintly yellow by iodine (p. 36).

ii. Swollen and ultimately dissolved by sulphuric acid (p. 36).

iii. Coloured blue with iodine and sulphuric acid (p. 36).

iv. Coloured blue or violet with chlor-zinc-iodine (p. 37).

v. Swollen and dissolved by ammoniacal solution of cupric hydrate (p. 37).

vi. Stained by solutions of carmine or of hæmatoxylin which contain a mordant, by methylene blue, and in various degrees by other aniline colours.

Lignified Cell-walls.

i. Coloured distinctly yellow by iodine (p. 38), and by chlor-zinc-iodine (p. 39), but in the case of bast-fibres the tint may vary to sherry brown, or even pink (p. 95).

ii. Coloured brown and swollen by iodine and sulphuric acid.

iii. Coloured bright yellow by acidulated solution of aniline sulphate (p. 39).

iv. Coloured red with acid solution of phloroglucin (see Appendix A).

v. Coloured green when exposed to light after treatment with carbolic and hydrochloric acids (see Appendix A).

vi. Stained slightly or not at all by solutions of carmine, and hæmatoxylin, but readily by aniline colours.

Cuticularized or Corky Cell-walls.

i. Coloured yellow by iodine (p. 39).

ii. Coloured yellow or brown by chlor-zinc-iodine (p. 39).

iii. Coloured yellowish by strong potash: on gradually warming (without boiling), they become bright yellow: on boiling, yellow drops of suberin escape.

iv. They resist the action of sulphuric acid, retaining their clearly-marked outline (p. 39).

v. On treatment with Schulze's macerating fluid, the cuticularized cell-walls become conspicuous: on boiling in it, their substance escapes as viscid drops of ceric acid (p. 39).

vi. They are dissolved slowly by strong chromic acid, but resist its action for some time.

vii. They are not stained by solutions of carmine or hæmatoxylin, but are coloured by aniline stains.

Mucilaginous Walls, resemble cellulose in many of their reactions.

i. They swell with water (p. 94).

ii. They swell to a greater extent with potash.

iii. They do not stain with iodine.

iv. They stain pink with corallin soda (p. 95).

v. They stain red with Hanstein's aniline-violet, blue with methylene blue; some kinds of mucilage also stain with Hoffmann's blue.

Callus is found on the plates of sieve-tubes.

i. It is soluble in sulphuric acid.

ii. It is stained by Hoffmann's blue, and by hæmatoxylin.

iii. Brown by Russow's callus-reagent (p. 115).

iv. Pink with corallin-soda (p. 115).

v. It is largely swollen by potash.

Mineral Deposits in cells or cell-walls.

A. **Silica.** If a tissue be ignited on platinum foil (after soaking in nitric acid, or Schulze's macerating fluid), and the ash, after being treated with acetic or nitric acid, shows an insoluble residue, the residue is silica (p. 90).

B. **Calcium Oxalate** occurs in the form of crystals (p. 109).

i. Insoluble in acetic acid.

ii. Soluble without evolution of gas in nitric acid.

iii. Soluble in sulphuric acid, with formation of fresh crystals of calcium sulphate, if only small bulk of fluid be present.

iv. Are not stained with iodine, &c.

C. **Calcium Carbonate** occurs as incrustations (p. 140), or crystals: it is soluble in acetic acid with evolution of bubbles of gas (CO_2).

Protoplasm or **Proteids** generally.

i. Coloured yellow or brown by preparations of iodine (pp. 26, 27).

ii. Coloured yellow by nitric acid: on the addition of potash or ammonia a bright yellow colour is produced (xanthoproteic reaction).

iii. Swells and loses details of structure on treatment with potash, ammonia, or "eau de javelle" (p. 49).

iv. Stains readily with solutions of carmine, hæmatoxylin, or Hoffmann's blue; bright red with Hanstein's aniline violet.

The best stains for the nucleus, and for showing the details of its structure, are hæmatoxylin, safranin, and methyl-green.

Plastids show under favourable circumstances the same reactions as other proteid bodies.

Aleurone-grains and **crystalloids** give also the characteristic reaction of proteids (p. 223). There is a considerable variety in the solubility of these bodies in water, or in salt-solution, in different seeds: the following will serve as types:—

1. *Grains without crystalloids.*

a. Soluble in water: peony, almond, cherry, apple.

b. Partially soluble in water; more or less readily soluble in 10 per cent. solution of common salt.

α. Soluble in saturated solution of common salt: lupine, pea, bean, scarlet-runner.

β. Soluble in saturated solution of common salt only after treatment with alcohol: sunflower, turnip, cress.

2. *Grains containing crystalloids.*

a. Partially soluble in water; more or less readily soluble in 10 per cent. solution of common salt.

α. Soluble in saturated solution of common salt: Brazil nut, pumpkin.

β. Soluble in saturated solution of common salt only after treatment with alcohol : castor-oil plant, walnut.

In all cases a mass (globoid) of mineral matter remains behind after the solution of the grain : this is soluble in acetic acid. The sections should be examined in alcohol.

Starch-grains.

i. Coloured blue with solutions of iodine in presence of water (p. 45).

ii. They swell in solution of potash (p. 49).

iii. They swell in water above 65° C.

iv. They swell in dilute sulphuric acid.

v. They swell and are coloured blue with iodine in chloral-hydrate (pp. 46, 130).

vi. They stain pink in corallin-soda solution.

Inulin.

i. Soluble, but not readily, in cold water.

ii. Precipitated as sphere-crystals on extraction of water by alcohol or glycerine (p. 120).

iii. Not appreciably coloured with iodine.

iv. Soluble, without coloration, in potash (p. 120).

v. Coloured an orange-red with alcoholic solution of orcin, after warming with hydrochloric acid.

Grape-Sugar.

i. Soluble in water.

ii. Less soluble in alcohol (p. 221).

iii. Gives a bulky yellow precipitate with Fehling's solution.

Cane-Sugar differs from the above in giving no precipitate with Fehling's solution.

Asparagin.

i. Soluble in water.

ii. Precipitated by alcohol (p. 228).

iii. Distinguished from other bodies which give the above reaction by insolubility in a saturated solution of asparagin.

Fixed Oils.

i. Coloured black with osmic acid.

ii. Saponified more or less readily by potash (p. 223).

iii. Soluble in ether.

iv. Stained pink by alkanna.

v. Some fixed oils are soluble in alcohol: *e.g.* oil of *Ricinus* (p. 223).

Caoutchouc.

i. Swollen, but not dissolved, by potash.

ii. Stained with tincture of alkanet.

iii. Soluble in chloroform or benzol.

Tannin.

i. Coloured deep brown by potassium bichromate, or chromic acid.

ii. Coloured greenish-blue by solution of ferrous sulphate and nitric acid.

iii. Gives a bulky yellow precipitate with solution of ammonium molybdate in strong solution of ammonium chloride.

Resin.

i. Soluble more or less readily in alcohol, or ether.

ii. Coloured red by alkanna (p. 66).

iii. Coloured blue by Hanstein's aniline-violet.

APPENDIX C

LIST of material required, together with notes on its preparation, the time of year at which the specimens should be taken, and the pages on which they are severally mentioned in the text, &c. Specimens marked with an asterisk (*) are required for the work described in large type.

**Abies excelsa:* not native, but commonly grown as an introduced plant; the whole shoot, at any time of year, p. 229.

**Acacia*, various species: botanic gardens; phyllodes, p. 134.

Acer Psendo-Platanus, common Sycamore: seed, winter, p. 216.

Acetabularia, a genus of Siphonaceous Algæ: specimens may be obtained from the coasts of the Mediterranean (p. 436).

**Æcidium* (see *Puccinia*).

**Æsculus Hippocastanum*, the Horse-chestnut, roots of various ages; 153.

**Agaricus campestris*, the common Mushroom: may be collected in the autumn, or it may be cultivated (p. 453) from the "spawn," which is sold by nurserymen in bricks, in which case specimens may be obtained at any time of year. For directions how material should be prepared, see p. 456.

**Alisma Plantago:* native, in watery places common; the young fruits for development of embryo should be used fresh, or may be hardened in alcohol (July–August), p. 211.

**Alkanna*, or Alkanet: root of *Anchusa tinctoria*, may be bought dry from druggists, p. 65.

**Almond:* to be bought from grocers; p. 216.

Anthoceros lævis, or *punctatus*, native species of Liverworts: not common: to be found growing on moist ground: the sporogonia are ripe in July: to be used fresh or in spirit (p. 378).

**Apple:* some mealy-fleshed kind (*e.g.* Baldwin Pippin); may be bought almost all the year round; (p. 25).

Araucaria imbricata, or other species: introduced; the whole shoot; (p. 230).

Ascobolus, a Discomycetous Fungus, which grows on dung if kept under a bell-glass (p. 472).

**Aspergillus* (see *Eurotium*).

**Aspidium Filix-mas*, a common native Fern: to be taken in late summer; whole plant, fresh, 287; stem, fresh, 289; apex of stem, fresh, or, better, hardened in spirit, 300; root, 303; apex, 305.

Batrachospermum, a native genus of Algæ, found growing attached to stones in fresh-water streams: the plant is dark-coloured and slimy to the touch: to be used fresh if possible (p 384).

**Beta vulgaris*, the common Beet of gardens: the fresh root may be bought of greengrocers; (p. 32).

**Begonia:* any of the hot-house species will do; lamina, fresh (p. 139).

Boletus, a genus of native Agarics, recognized by the spongy hymenium; several species are commonly to be found in woods in the autumn (p. 460).

Botrydium granulatum, to be found occasionally growing on mud at the margins of ponds (p. 436).

Botrytis cinerea, a common mould which grows on dead parts of plants (p. 472).

Brassica oleracea, the common garden Cabbage: leaves, for chlorophyll-solution, p. 128.

Broad Beans (see *Vicia*).

Bryopsis plumosa, a Siphonaceous marine Alga, found growing on stones and rocks in summer, but not common (p. 436).

Bulbochæte, a genus of Confervoid Algæ: *B. setigera* is to be found not uncommonly growing in fresh-water (p. 424).

Buxus sempervirens, the common Box of gardens; leaf; 124.

Cabbage (see *Brassica*).

Callithamnion, a genus of red Seaweeds (*Florideæ*), including

many species commonly found between tide-marks on our coasts (p. 387).

**Caltha palustris*, the common Marsh Marigold of swamps: flower (April–May), fresh, 188; hardened in absolute alcohol, stamens, 200; carpels, 204; endosperm, 212.

Camellia japonica: leaf, 141.

Canna: commonly grown in gardens; rhizome, treated with picric acid, and alcohol, p. 220.

**Capsella Bursa-Pastoris*, the common Shepherd's Purse: fruits of various ages, summer, to be used fresh, 209.

Cerastium, any native species: young shoots, 57.

**Cetraria Islandica* (the "Iceland Moss"), a Lichen rarely found native in this country; but it may always be obtained from druggists in the dry state (p. 479).

Chætophora, a genus of fresh-water Algæ, which grow attached to stones, &c. (p. 425).

**Chara*, sp.: plants of this genus are common in fresh-water, fresh or in spirit, 409.

Cheiranthus Cheiri, the Wallflower: leaf, fresh or in spirit, (p. 127).

Cherry Laurel (see *Prunus Lauro-Cerasus*).

Chondrus crispus (Carrageen), a very common red Seaweed, to be found on all our coasts, attached to stones between tide-marks: to be hardened in spirit (p. 385).

Chrysanthemum Leucanthemum, common Ox-eye Daisy: capitulum, 197.

Citrus Aurantium, common Orange: young fruits, to be hardened in alcohol, 120.

Cladophora, sp.: green Algæ, of which some species grow in fresh, others in salt-water, 427.

**Claviceps purpurea*, the Ergot of Rye, to be found commonly on Rye, and other Grasses in autumn, causing a malformation of the ovary: the sclerotia may be bought from druggists (p. 481).

Clematis Vitalba, the native Traveller's Joy: stem, 57, 79.

**Closterium*, a genus of Desmids: frequently to be found in standing fresh-water: to be observed in the fresh state (p. 447).

Codium tomentosum, a marine Siphonaceous Alga, not

uncommon on our coasts: it grows attached to rocks near low-water mark (p. 435).

**Coleochæte*, a genus of Confervoid Algæ, which may commonly be found attached to the surface of submerged Phanerogams in fresh-water streams and ponds :to be used fresh if possible (p. 419).

**Collema*, a genus of gelatinous Lichens, of which various species are commonly to be found growing on moist soil, stones, &c.: to be hardened in alcohol before sections are cut (p. 475).

Coprinus, a genus of Fungi, which commonly grow on dung, or manured land: they may almost certainly be obtained by keeping fresh horse-dung under a bell-glass for two or three weeks (p. 460).

Corallina officinalis, the common Coralline Seaweed of all our coasts; found in large quantities in tidal pools, and recognized by its superficial deposits of lime (p. 385).

Correa, sp.: an exotic shrub, to be obtained in botanic gardens; hairs, 146.

*Cotton wool (*Gossypium*), 35; *orchis*, tubers of, 95.

**Cratægus Oxyacantha*, the common Hawthorn: flower, 190.

Crucibulum vulgare, one of the Gasteromycetous Fungi: to be found not uncommonly growing on sticks, and decaying Fern, &c. (p. 461).

**Cucurbita Pepo*, the Vegetable Marrow, or *Cucumis sativus*, the Cucumber, may be used indifferently: seedlings, fresh, 226; stems, 113.

Cupressus, sp.: root, 250.

**Cystopus candidus*, a Fungus, commonly found growing parasitically on the shoot of Shepherd's Purse, appearing as a "white rust" in late summer: may be preserved in alcohol, for use at other times of year (p. 495).

Cytisus Laburnum: old stem, 100.

Dædalea, a native genus of Hymenomycetous Fungi: the commonest is *Dædalea quercina*, which is to be found growing on oak stumps (p. 460).

Dacryomyces, a Fungus which grows on decaying wood, 461.

Dahlia: leaf; 128.

Dandelion (see *Taraxacum*).

*Date (*Phœnix dactylifera*) : the "stone," 38, 221, 228.

**Datura Stramonium*, a casual weed : the style and stigma, fresh, 207.

Davallia, sp. : rhizome, 292.

Dictyota dichotoma, a marine Alga, which grows fixed upon other Algæ, or on rocks, near low-water mark, in summer : not uncommon (p. 381).

**Digitalis purpurea :* flowers (July–September), of various ages, for fertilization, 208.

**Dracæna*, stem, 169.

Dudresnaya, a genus of marine Algæ, which grow in summer on rocks or other Algæ near low-water mark ; *D. divaricata* is not uncommon (p. 389).

Ebony (*Diospyros*, sp.) : old stem, 100.

Elæagnus, sp. : leaf, fresh, for hairs, 146.

*Elder (see *Sambucus*).

*Elm (see *Ulmus*).

**Elodea canadensis :* the common American weed ; an introduced plant, to be found universally in still, fresh-water ; to be used fresh ; stem, 171 ; leaf, 180.

**Equisetum arvense :* a common corn-field and road-side weed ; whole plant, which may be obtained throughout the summer, and to be used fresh or in alcohol, 325 ; buds, hardened in alcohol, 334 ; fertile stems, with sporangia, to be collected in spring, 338 ; spores, to be sown in spring, 340.

Equisetum Telmateia : mature stem, summer, 330.

**Eucalyptus globulus*, the Blue Gum of Australia : common in green-houses, leaf, 135.

**Euphorbia splendens* (a commonly grown exotic), or other species of *Euphorbia :* fresh stem, 120.

**Eurotium Aspergillus*, a mould common on old leather, decaying fruit, &c., and may be obtained without fail if a slice of dry bread be kept under a bell-glass for ten days or more (p. 484).

Fegatella conica, a Liverwort not uncommon on river banks, (p. 367).

*Fern prothalli are to be found growing on surfaces of pots, &c. in any fern-house, or they may be cultivated on damp soil, or on cork floating in water, from the fresh spores : they may be used

either fresh, 30, 47, 311, or hardened in alcohol, or in picric acid and alcohol, 130, 314, &c.

**Ficus elastica*, the India-rubber plant: grown commonly in green-houses, and dwelling-houses; the leaf may be used fresh or in spirit, 139.

**Fritillaria imperialis:* a common garden plant, flowering in early summer; nectaries, 199; pollen, 203; endosperm, 213.

Fuchsia: leaf, hardened in alcohol, for water-stomata, 141.

**Fucus serratus*, the common serrate Wrack of all our coasts: found chiefly about half-tide level: material to be hardened in alcohol before use: the whole thallus is required, including the organ of attachment. The male and female organs are on different plants, and may be distinguished by their colour with the naked eye, (p. 391). *F. platycarpus* and *F. vesiculosus* are also common all round the coast (p. 393).

**Funaria hygrometrica*, a common native Moss, frequently to be found on charred or burnt soil: the whole plant is required, and should be hardened in alcohol (p. 342, &c.).

Geaster, a genus of rather uncommon Gasteromycetous Fungi, 461.

Geranium, Scarlet (see *Pelargonium*).

**Glœocapsa*, a genus of gelatinous Algæ, belonging to the Cyanophyceæ: it grows on wet rocks, and is commonly found forming a film on the inner surface of the glass of damp conservatories (p. 452).

Gomphonema, a genus of stalked Diatoms, commonly to be found attached to the surface of submerged fresh-water plants, 449.

*Grapes: ripe, fresh, 221.

Graphis, a genus of Crustaceous Lichens, commonly found on the smooth bark of trees, and appearing as irregular dark lines breaking through the surface of the bark (p. 479).

Hakea suaveolens: exotic shrub; leaf, 140.

Hedera Helix, the common Ivy: stem, fresh, 65.

**Helianthus annuus*, the Sunflower: ripe fruits, to be obtained from seedsmen, 216; whole plants, 54; stem, fresh or in spirit, 61; young stem, fresh or in spirit, 76; apical bud, hardened in spirit, and taken in early summer, 79; leaf, fresh or in spirit, 122; root, 148; root apex, from dry fruit, 157; mature capitulum, fresh or

in spirit, 191 ; young capitulum, taken in early summer, and hardened in spirit, 194.

**Helianthus tuberosus :* tuber, after treatment with alcohol, 119 ; apical bud, in spirit, 83.

Hieracium Pilosella, a common native Hawkweed : leaf, for hairs, 146.

Himanthalia lorea, a marine Alga, usually growing on flat rocks about half-tide level : it may be recognized by the cuplike base of the thallus, and thong-like dichotomous upper branches : the apex is to be hardened in alcohol before use (p. 400).

**Hippuris vulgaris*, the common Mare's-tail : found growing in water ; stem, fresh, 110 ; apical bud, fresh, 111 ; leaf, 130 ; scale-hairs, 146.

Holly (*Ilex Aquifolium*) : leaf, fresh or in spirit, 130.

*Horse-chestnut (*Æsculus Hippocastanum*) : leaf, to illustrate fall and scar, autumn, spirit, 144 ; colleters, 146 ; root, fresh or in spirit, 153.

Hoya, sp. : leaf, fresh or in spirit, 141.

Humulus Lupulus, the common Hop : glandular scales, 146.

**Hyacinthus orientalis*, the garden Hyacinth : whole plant, 167 young scape, in spirit, 86 ; scape or leaves, 177 ; scape, 168 ; leaf, 175 ; root, spirit, 180.

**Hydrocharis Morsus-Ranæ :* a floating plant, native, but not common, from Northumberland southwards ; root, fresh, 33.

Hydrodictyon utriculatum, a somewhat uncommon fresh-water Alga, which has the form of a reticulate sac (p. 441).

Hydnum, a genus of Hymenomycetous Fungi, found in woods 460.

Hymenophyllaceæ : stems, 292.

Iberis amara, the Candy Tuft : common in gardens ; young shoots, 57.

**Iris Pseud-Acorus*, the native yellow Iris : leaf, 179 ; flower, 193.

Ivy (see *Hedera*).

Jerusalem Artichoke (see *Helianthus tuberosus*).

Juglans regia, the Walnut : fruit, 216.

Juniperus, sp. : shoot, 230 ; phloem, 237.

Jungermannia, a genus of Liverworts, including many common

species, found growing on moist soil or stones, in wet places (pp. 363, &c.).

Kidney Bean (see *Phaseolus*).

Laburnum (*Cytisus Laburnum*) : old stem, 100.

Laminaria digitata, a large brown Seaweed, found attached to rocks near or below low-tide mark : the parts required are to be hardened in alcohol before use (p. 406).

Lavatera arborea : an uncommon native plant, cultivated frequently in gardens ; hairs, 146.

Lemanea, a genus of fresh-water Florideæ, found growing attached to stones, in swiftly-flowing mountain streams (pp. 388, 386).

Lilium, sp. : ovary, 206 ; leaf, 176.

**Linum usitatissimum :* seed (Linseed), to be bought in shops, 94 ; apex of root, 159.

Lunularia vulgaris, a very common Liverwort, resembling *Marchantia*, but distinguished by the semilunar cups in which the gemmæ are produced (p. 361).

**Lupinus luteus :* seedlings may be raised from seeds to be obtained from seedsmen ; 228.

**Lychnis dioica*, the common Rose Campion : flower, 189.

Lycoperdon (Puff-ball), a common genus of Gasteromycetous Fungi, which grow for the most part on grass-land (p. 461).

**Lycopodium clavatum*, the native "Stag's-horn Moss" : to be found on moorland hills : to be used fresh or in spirit, 275, &c.

Maize (see *Zea*).

**Marchantia polymorpha*, a common Liverwort, to be found growing in moist places, on flower-pots in green-houses, &c. : the whole plant is required, together with male and female branches : the material is to be hardened in alcohol, or may be used fresh (p. 360, &c.).

Marsilea, sp. : commonly grown in botanic gardens ; 324.

Mesocarpus, a genus of filamentous fresh-water Algæ (p. 446).

Metzgeria, a native Liverwort ; apex of thallus, 367.

Micrasterias, a genus of Desmids, commonly to be found in peat bogs (p. 448) : should be observed while living.

**Mirabilis Jalapa :* ripe fruits, may be obtained from seedsmen ; 217.

Morchella (the Morel), a native Fungus of variable occurrence, found especially upon soil which has been burnt (p. 471).

**Mucor*, various moulds of this genus are found growing on organic substrata, and may be readily obtained on wet bread, or dung kept under a bell-glass (p. 497) : should be used fresh.

Navicula, a very common genus of Diatoms, found in fresh-water, and capable of slow movement (p. 449).

Nettle (see *Urtica*).

Nitella, sp. : plants of this genus are common in fresh-water : fresh or in spirit, 409, &c.

**Nostoc*, a dark olive-coloured gelatinous Alga, belonging to the Cyanophyceæ : commonly to be found on lawns, and among moss : swelling greatly with water, it often comes first into notice after rain (p. 451). : should be hardened in alcohol for use.

Nymphæa alba, native white Water-Lily : leaf, 139.

Nuphar lutea, native yellow Water-Lily : leaf, 139.

Oak (see *Quercus*).

**Œdogonium*, a genus of green filamentous Algæ, growing attached to stones, or submerged plants in fresh-water: to be recognized by the peculiar intercalary growth, and the irregular thickness of the filaments (p. 421).

Oranges (see *Citrus*).

**Oscillatoria*, a genus of blue-green filamentous Algæ, commonly to be found growing on moist soil, stones, &c. (p. 452).

Pandorina Morum, a green Alga, consisting of a motile spherical colony of sixteen cells : not uncommon in ponds : should be observed while actively living (p. 439).

**Parmelia parietina*, the common yellow Lichen, found on walls, roofs, rocks, &c. : may be dried for subsequent use (p. 473).

Pelargonium, or Scarlet Geranium : petals, fresh, 198 ; leaf, or stem, fresh, 146.

**Pellia epiphylla*, a common native Liverwort : thallus, 362 ; antheridia, 371 ; sporogonium, 377.

Peltigera canina, a common Foliaceous Lichen, which grows on a mossy substratum (p. 479).

Penicillium, the common blue mould, 490.

Peperomia : various species, which are grown in botanic gardens will do ; leaf, fresh, 139.

**Peziza*, a large genus of variously coloured, cup-shaped Fungi, to be found growing in various positions, *e.g.* on rotting wood, moist soil, &c.: to be hardened in alcohol for use (p. 470).

Phallus impudicus, a Gasteromycetous Fungus, found commonly growing in woods (p. 461).

**Phaseolus vulgaris*, the Kidney Bean: seedlings, 226; root 148; apex of root, 159.

Phaseolus multiflorus, the Scarlet-Runner: pulvinus, fresh or in spirit, 123.

Phajus grandifolius, an exotic Orchid: young tubers, 221.

Phyllocladus: a southern Conifer; botanic gardens; shoot, 230.

Pilobolus, a Fungus which appears upon cow-dung, if kept under a bell-glass, 499.

**Pilularia globulifera:* a native aquatic, not uncommon in heathy marshes; whole plant, fresh or in spirit, 319.

Pinnularia, a genus of not uncommon Diatoms (p. 449).

**Pinus sylvestris*, the common Scotch Fir: wood, 233; shoot, 236; stem, in spirit, 232; leaf, in spirit, 244; roots, of various ages, in spirit, 247; male inflorescence, fresh or in spirit, May and June, 252; female inflorescence, spirit, to be taken at various periods from beginning of June to the autumn, 254.

Pisum sativum: dry seeds, for apex of root, 160.

**Pleurococcus* constitutes the greater part of the green incrustation on the bark of trees: to be used fresh (p. 439).

Poinsettia: a common green-house plant; inflorescence, 142.

Polygonum Fagopyrum, the Buckwheat: apex of root, 159.

Polyides, a purple marine Alga, not uncommon in rocky pools, between tide-marks (p. 389).

Polyporus, a genus of Hymenomycetous Fungi, which grow on decaying wood (p. 460).

**Polysiphonia fastigiata*, a red Seaweed, which may constantly be found growing attached to the thallus of *Ascophyllum nodosum* on all our coasts: may be examined fresh or in spirit (p. 379).

**Polytrichum commune*, a Moss, common on moors, &c.: may be used fresh, or better, after hardening in alcohol, p. 341, &c.

**Potamogeton*, sp. : a common genus of native aquatics ; fruit in various stages of development, to be preserved at various times in summer, in spirit, 213.

Potato (see *Solanum*).

Prunus Lauro-Cerasus, the common Cherry-Laurel of gardens : leaf, fresh or in spirit, 134 ; glands on leaf, 142.

**Prunus Padus*, Bird Cherry : flowers, 189.

Primula sinensis, the common green-house *Primula :* leaf, 146 ; flower, 190.

**Primula vulgaris*, the common Primrose : flower, 190.

**Pteris aquilina*, the Bracken Fern : apex of rhizome, 303 ; rhizome, in spirit, 298.

Ptilota, a genus of red Seaweeds, found growing on vertical rock faces, between tide-marks, or on stems of Laminarias : to be used fresh, if possible (p. 385).

**Puccinia graminis* (*Æcidium Berberidis*), the rust of Wheat, and other Grasses : is now less common than formerly, but may frequently be found as dark streaks on the leaf and stem of Grasses ; while the æcidium forms bright-coloured swollen patches on the leaves of *Berberis :* to be used fresh, or after hardening in alcohol (p. 462).

**Pythium de Baryanum* appears constantly on seedlings of Cress, which are grown close together, and kept very damp (p. 491).

**Ranunculus acris*, the common Buttercup : flower, 187.

Ranunculus, sp. : stem, 79.

Raphanus sativus, the garden Radish : root, 153.

Rhododendron ferrugineum, Swiss Rhododendron : often grown in botanic gardens ; 146.

**Rhododendron persicum*, the common Rhododendron of gardens : pollen-tubes, fresh, 208.

Riccia fluitans, a not uncommon Liverwort, found in fresh-water pools, &c., together with Lemna, &c. (p. 362).

**Ricinus communis*, the Castor Oil plant : ripe seeds may be obtained from seedsmen, 216 ; young stem, 79.

Robinia Pseud-Acacia : stem, 75.

Rosa, sp. : mature stems, 97 ; prickles, 147.

Rubus, sp. : mature stems, 97 ; prickles, 147.

Sambucus niger, the common Elder: young stems, May or June, spirit, 85.

Saxifraga, various species: leaves, for chalk glands, 142.

**Scilla nutans*, the wild Hyacinth: flower, 193.

**Sedum acre*, the common Stonecrop: leaf, fresh, 136.

**Selaginella Martensii:* commonly cultivated as "*Lycopodium*" in green-houses; whole plant, 261, &c.

**Solanum tuberosum*, the Potato: tuber, fresh, 44; young, and treated with picric acid and alcohol, 220.

Sphagnum, the genus of Bog Mosses, common on every moor: to be used fresh, or in alcohol (p. 357).

**Spirogyra*, a genus of floating, filamentous, fresh-water Algæ, common in still ponds or streams, and slimy to the touch: to be used fresh, if possible (p. 442).

**Sporodinia*, a Fungus which grows parasitically on the larger Agarics, and not uncommonly found on *Boletus* in autumn (p. 500).

Stachys, sp.: shoot, 57.

**Stellaria Holostea*, the common Stitchwort: flowers, in spring, 188.

**Stellaria media*, the Chickweed: fresh ovaries, various ages, summer, 206.

**Strychnos Nux-vomica:* seeds may be obtained from druggists; 214.

Sunflower (see *Helianthus*).

Sycamore (see *Acer*).

**Syringa vulgaris*, the common Lilac: winter; buds, 83.

**Taraxacum officinale*, the common Dandelion: root, preserved in spirit, 118; capitulum, 193.

**Taxus baccata*, the common Yew: shoot, 230; leaf, 247; root, 250; reproductive organs, in spirit, 259.

Thuja: shoot, 230.

Tilia europæa, the Lime-tree: four-year-old stem, 166.

*Tomato: ripe fruit, fresh, 199.

Tradescantia, sp.: commonly grown in botanic gardens; staminal hairs, fresh, 84.

Trianea bogotensis: an exotic floating plant, grown in botanic gardens; roots, with hairs, 33.

Tropæolum, sp. : fresh petals, 199 ; leaf, 141.

Tulip : of gardens and conservatories ; flower, 198.

**Ulmus campestris*, the Elm : stems of various ages, young twigs taken at end of May, 188 ; older stems, 97 ; winter twigs for leaf-scars, 143.

**Ulothrix*, a filamentous, fresh-water Alga, commonly found in early summer attached to stones on the margins of ponds : to be used fresh if possible (p. 424).

Urtica urens, the Nettle : leaf, or stem, 145 ; root, 153.

Usnea barbata, a fruticose Lichen, to be found especially in Fir woods, and growing attached to the stem and branches of trees 475 ; to be used dry.

Vallisneria spiralis : an exotic aquatic, often grown in aquaria ; leaves, 48.

Vanda, sp : an exotic Orchid ; aërial roots, 185.

**Vaucheria*, a genus of Siphonaceous Algæ : various species are to be found growing on moist soil, on the surface of flower-pots in conservatories, &c., or in still fresh-water : to be used fresh, 429.

Verbascum, sp. : native species of Mullein ; hairs, 146.

Viburnum Opulus, the Guelder Rose : glands on leaf, 142.

**Vicia Faba*, the Broad Bean : seed, 215 ; seedlings, 226 ; glands on stipules, 142.

Vitis vinifera, the Vine : stem, 101.

**Volvox globotor*, a green, fresh-water Alga, consisting of spherical, mobile colonies : not uncommon in fresh-water ponds : to be used fresh (p. 437).

Yucca, sp. : an arborescent Monocotyledon, commonly grown in gardens ; stem, 169 ; ovary, 206.

Zea mais, the Maize or Indian Corn ; fruits, fresh, to be bought of seedsmen, 218 ; germination, 227 ; stem of well-grown plant, fresh or in spirit, 161 ; leaf, 172 ; root, 180 ; apex of root, hardened in spirit, 183.

INDEX

Abciss layer, 145
Abies excelsa, 229
Abies, leaves of, 230
Abstriction, 436
Acacia, phyllode of, 134
Acetabularia, 436
Achænium, 216
Achromatin, 43
Adhesion, 191
Æcidiomycetes, 462
Æcidium, 465
Æcidium-spores, germination of, 467
Aërial roots, 185
Æsculus, leaf-scar of, 144 ; colleters of, 146 ; root of, 153
Agaricus, 452
Aggregated inflorescences, 194
Air-bubbles, 15
Air-cavities, *Marchantia*, 364
Alburnum, 100
Aleurone-grains, 223
Alkanna root, use of, 66
Almond, seed of, 216
Aloe, 42
Amphigastria, 361, 365
Amorphous coat of aleurone-grains, 224
Anatropous ovule, 205
Androecium, 188, &c.
Angiosperms, 54
Annual rings, of Elm, 98 ; of Pine, 239 ; of root, 250
Annulus of Fern, 310 ; of Moss, 353 ; of Mushroom, 455
Anther, 200, 253
Antheridia, of Fern, 313, 316 ; of *Equisetum*, 340 ; of Moss, 341, 347; of *Marchantia*, 361, 370 ; of *Polysiphonia*, 388 ; of *Fucus*, 401; of *Chara*, 415 ; of *Coleochæte*, 420 ; of *Œdogonium*, 424 ; of *Vaucheria*, 431 ; of *Volvox*, 438; of *Pythium*, 494 ; of *Cystopus*, 496
Antheridiophore, 369
Antherozoids, see Spermatozoids
Anticlinal walls, 80, 82, 302
Antipodal cells, 206
Apex, of stem, Sunflower, 79 ; of stem, *Hippuris*, 111 ; of root, Sunflower, &c., 157 ; of stem, Maize, 166 ; of root, Maize, 183 ; of root, Pine, 251 ; of stem, *Selaginella*, 269 ; of stem, *Lycopodium*, 282 ; of stem, *Aspidium*, 301 ; of stem, *Pteris*, 303 ; of root, Fern, 305 ; of prothallus, Fern, 313 ; of stem, *Equisetum*, 334 ; of root, *Equisetum*, 338 ; of stem, of Moss, 344 ; of *Marchantia*, 366 ; of *Polysiphonia*, 380 ; of *Fucus*, 399 ; of *Chara*, 413
Apophysis, 342
Apothecium, 470, 473, 477
Apparatus, 1
Apple, 25
Aquatic type of Dicotyledon, 110 ; of Monocotyledon, 171
Araucaria, leaves of, 230

Arboreous type of Dicotyledon, 88 ; of Monocotyledon, 169
Archegoniophore, 372
Archegonium, of Pine, 357 ; of Fern, 313, 315 ; of Moss, 349 ; of *Marchantia*, 373, 374
Archesporium, 203, 272, 285, 311, 353
Archicarp, 488
Arillus, 217, 260
Artichoke, inulin of, 119
Ascobolus, 472
Ascogone, 479
Ascomycetes, 470
Ascophyllum, 379
Ascospores, 489
Ascus, of *Peziza*, 471 ; of Lichens, 477 ; of *Claviceps*, 483 ; of *Eurotium*, 489
Asparagin, 228
Aspidium Filix-mas, 287
Auxospores, 450
Azygospores, 502

Bark, 101
Basidia, 459, 466
Basidiomycetes, 453
Bast-parenchyma, 95, 103, 236, &c.
Batrachospermum, 384
Beet-root, plasmolysis, 32 ; sugar of, 222
Begonia, crystals of, 179 ; leaf, multiple epidermis of, 139
Berberis, 465
Bird cherry, flower of, 189
Blasia, 367
Blue-bell, flower of, 193
Boletus, 460
Bordered pits, 237, 240, 297
Botrytis, 470, 472
Bracken, rhizome of, 298
Bract, 191
Bracteole, 191, 195
Broad Bean, 215
Bryophyta, 341
Bryopsis, 436
Bulbochæte, 424
Bulbous type of Monocotyledon, 167
Bundles, bi-collateral, 114
Bundle-sheath, Sunflower stem, 66; *Hippuris*, 110 ; of root, 150 ; of *Selaginella*, 264 ; of *Lycopodium*, 277 ; of Ferns, 295 ; of Fern root, 304 ; of *Equisetum*, 331 ; see also endodermis
Bundle-system, method of investigating, 59 ; of *Lycopodium*, 280 ; of Ferns, 290 ; of *Equisetum*, 330
Buttercup, flower of, 187
Buxus, leaf of, 124

Calcium carbonate, 140, 142
Calcium chloride, clearing by, 50
Calcium oxalate crystals, 109, 178
Callithamnion, 387
Callus, 103, 115
Caltha, flower of, 188 ; stamen of, 200 ; carpel and ovules of, 204 ; development of endosperm of, 212
Calyptra, of Mosses, 342, 351, 359
Calyptrogen, 160
Calyx, 187, &c.
Cambiform cells, 73, 114, 164
Cambium, of Sunflower stem, 73 ; interfascicular, 176 ; of Elm, 95, 98, 106 ; of root, 152 : of Pine, 235 ; of *Laminaria*, 407
Camellia, sclerenchyma of leaf, 141
Camera lucida, 21
Campylotropous ovules, 209
Canada balsam, 52
Canal cell, 315, 349
Cane-sugar, 222
Capitulum of Sunflower, 191 ; development of, 194
Capsella, embryo of, 209 ; white rust of, 495
Carpels, 204, &c.
Carragheen, 385
Caulerpa, 436
Cauline bundles, 112, 169
Cell-division, 84 ; of *Spirogyra*, 444
Cell-plate, 85
Cell-sap, 32 ; substances in solution in, 198
Cell-walls, 25, 27, 30
Cellulose walls, reactions of, 35
Central nucleus of embryo-sac, 206

Cerastium, bundle-system, 57
Ceric acid, 40
Cetraria, 479
Chalk-glands, 142
Chantransia, 386
Chara, 409–418
Cherry Laurel, leaf of, 134 ; glands of, 142
Chlamydomonas, 440
Chlorophyll-corpuscles, 30, 47, 128; division of, 47
Chlorophyll, solution of, 128 ; spectrum of, 129
Chloroplasts, 30, 47, 128, &c.
Chlor-zinc-iodine, 3, 27, 39, &c.
Chondrus, 385
Chromatin, 43, 85
Chromatophores, 28 ; of *Coleochæte*, 420 ; of *Œdogonium*, 422 ; of *Ulothrix*, 425 ; of *Volvox*, 437 ; of *Pleurococcus*, 440; of *Spirogyra*, 444 ; of *Closterium*, 447
Chromoplasts, 199
Chrysanthemum, capitulum of, 197
Cilia, 314, 348, 375, &c.
Circinate vernation, 288
Circulation of protoplasm, 34
Cladophora, 427
Claviceps, 481
Clearing preparations, 49
Cleistocarpous Ascomycetes, 484
Clematis, 40 ; bundle-system of, 57, 60 ; interfascicular cambium, 79
Closterium, 447
Club-Moss, 275
Coagulum of latex, 118
Codium, 435
Cœnobium, 437
Cohesion, 189
Coleochæte, 419
Coleorhiza, 227
Collema, 475, 479
Collenchyma, 64, 71
Colleters, 146
Columella, of Mosses, 352 ; of *Sphagnum*, 359 ; of *Anthoceros*, 378 ; of *Mucor*, 499
Common salt, 29
Companion cells, 68, 114
Concentric bundles, 294
Conceptacle, 393, 401
Conducting tissue, 207
Confervoideæ, 419
Conidia, of *Aspergillus*, 485 ; of *Pythium*, 493 ; of *Cystopus*, 495
Conidiophores, 472, 485
Conjugation, of *Ulothrix*, 426 ; of *Spirogyra*, 446
Conjunctive parenchyma, 265, 295
Connective, 200
Contents of embryo-sac, 206
Contents of pollen-grain, 202
Continuity of protoplasm, 213
Coprinus, 456, 460
Corallina, 385
Cork, 88, 91, &c.
Cork-cambium, 91, &c.
Corky walls, reactions of, 39
Corolla, 187, &c.
Corpusculum, 257
Cortex, 63, 89, 150, &c.
Cotton-wool, 35
Cotyledons, 211, 215, 225, 227, 257, 318
Cratægus, flower of, 190
Cress, 491
Crucibulum, 461
Crystals, 93, 178, &c.
Crystalloïds, 225
Cucumber, seed of, 215
Cucurbita, chromoplasts of, 199 ; sieve-tubes of, 113
Culture, of Fungi, 484 ; of spores of *Mucor*, 499
Cupric hydrate, 37
Cuticle, 63, 126, 131, &c.
Cuticularized layers, 131
Cutting sections, 7
Cyanophyceæ, 451
Cystidia, 460
Cystocarps, 379, 389
Cystoliths, 140
Cystopus, 495

Dacryomyces, 461
Dædalea, 460
Dammar, 52
Damping off, 491
Dandelion, 118, 193
Date, endosperm of, 38, 221; germination of, 228

Datura, 207
Davallia, stem of, 292
Dehiscence, of anther, 201 ; of sporangia, 310 ; of sporogonium, 353
Dermatogen, 81, 159, &c.
Desmidieæ, 447
Diaphragms, *Hippuris*, 111
Diatomaceæ, 449
Dichotomy, 360, 393, 381
Dicotyledons, vegetative organs of, 54, &c.
Digitalis, 208
Dimorphic flowers, 191
Dicœcious plants, 189
Directions of section, 7
Discomycetes, 470
Disk florets, 192
Dracænæ, stem of, 169
Drawing, 20
Duramen, 100

Eau de javelle, 50
Ectoplasm, 28, 433
Egg-cell, see Ovum
Elaters, 339, 376
Elder, pith of, 44
Elm, external characters of shoot, 88; stem of, 88–109 ; leaf-scar of, 143
Elodea, 34; stem of, 171; leaf of, 180
Embedding, 10
Embryo, mature, 215 ; of Maize, 218 ; development of, in Angiosperms, 209 ; in *Pinus*, 257 ; in *Selaginella*, 273 ; in Ferns, 317
Embryo-sac, 205, 255, &c.
Embryonic cell, 210
Emergences, 145
Endodermis, 150, 181, 185, &c. ; see also Bundle-sheath
Endoplasm, 28, 434
Endosperm, 212, 217 ; of Maize, 219 ; of *Pinus*, 257
Epidermis of Sunflower, 62, 127 ; of Holly, 131 ; of Elm, 90 ; of Maize, 162, 173 ; of Hyacinth, 174 ; of Pine, 245 ; of *Selaginella*, 363 ; of *Lycopodium*, 273, 276, 282 ; of Fern, 307 ; of *Equisetum*, 332
Epipetalous stamens, 190
Epiphragm, 342
Epispore, 496
Epithelium of resin-passages, 72
Epithema, 141
Equisetineæ, 325
Equisetum, 325–340
Ergot, 481
Eucalyptus, leaf of, 135
Euphorbia, laticiferous cells, 120
Eurotium, 484
Excipulum, 478
Exospore, 273
Exodermis, 180, 182, 185
Extension of internodes, 83
Extraction of embryo, 209
Extra-floral nectaries, 142

Fegatella, 367
Female branches (*Marchantia*), 372
Female cones, Pine, 254
Fertilization, 206, of Fern, 317 ; of *Marchantia*, 375 ; of *Vaucheria*, 432 ; of *Fucus*, 405
Ficus elastica, epidermis of leaf, 139 ; cystoliths of, 140
Fibrous cells or fibres, 104
Fibrous thickening of wall of anther, 201
Filament, 188
Filicineæ, 187, &c.
Floral receptacle, 187
Florets, 191
Florideæ, 379
Flower, 187 ; origin and development of, 194
Foliar gap, 290
Foot, 273 ; of Fern, 317
Fritillaria, nectaries of, 199 ; mature pollen of, 202
Fruticose Lichens, 475
Fuchsia, water stomata of, 141
Fucus, 91
Funaria, 346 ; sporogonium of, 351
Fungi, 453
Funiculus, 205

Gametangia, 435
Gametes, 428, 501
Gasteromycetes, 461

Geaster, 461
Gelatinous Lichen, 475
Gelatinous sheath, *Spirogyra*, 443
Gemmæ, 356, 368; of *Mucor*, 500
Geranium, see *Pelargonium*
Germination, of *Helianthus*, 225; of Maize, 227; of Pine, 258.
Gills of Mushroom, 455
Glands, 142
Glandular epithelium, 143
Glandular hairs, 146; internal of Fern, 293
Globoid, 224
Globule of *Chara*, 415
Glœocapsa, 452
Glycerine, 2, 26; permanent mounting in, 51
Glycerine jelly, 52
Gomphonema, 449
Gonidia of Lichens, 474
Gonidial layer, 474
Gonidiophore, 483; of *Mucor*, 497
Gossypium, 35
Grape-sugar, 221
Graphis, 479
Ground-tissue, 293
Guard-cells of stoma, 127, 133, 175, 177, &c.
Gymnosperms, 229
Gynœcium, 188

Hairs, various forms of, 145
Hakea, 140
Hardening, 4
Haustoria, 496
Hawthorn, flower of, 190
Hedera, resin-passages of, 72
Helianthus tuberosus, inulin of, 119
Helianthus annuus (Sunflower), vegetative organs, 54; stem, 55; leaf, 122; root, 148; capitulum, 200
Hepaticæ, 360
Hesperidin, 120
Heterocysts, 451, 475
Heterœcism, 467
Heteromerous Lichen, 474
Heterosporous type, of Lycopod, 261; of Fern, 319
Hilum, 44, 217
Himanthalia, 400
Hippuris, stem of, 110; apex of, 111; leaf of, 138; hair of, 146
Holly, leaf of, 130
Homoömerous Lichen, 475
Homosporous type, of Lycopod, 275; of Fern, 287
Hormogonia, 451
Horse-tail, 325
Hoya, 141
Hyacinthus, protoplasm and nucleus, 42; raphides, 178; external conformation, 167; development of stoma, 177; leaf, 175: root, 181
Hydnum, 460
Hydrocharis, 33
Hydrodictyon, 441
Hymenial layer, 459
Hymenium, of Mushroom, 459; of *Æcidium*, 466; of *Peziza*, 471; of Lichen, 477
Hymenophyllaceæ, stem of, 292
Hyphæ, 345, &c.
Hypoderma, 132, 245
Hypophysis, 210

Iberis, bundle-system of, 57
Iceland Moss, 479
Idioblasts, 132
Included starch-grains, 129
Indusium, 309
Inferior ovary, 192
Inflorescence, 187, 194
Initial cells, of *Lycopodium*, 283; of *Marchantia*, 367; of *Fucus*, 392
Integument, 205, 256
Intercalary growth, of *Anthoceros*, 378; of *Œdogonium*, 422
Intercellular spaces, 65, &c.
Interfascicular cambium, 78
Internal glandular hairs of Fern, 293
Internal hairs of *Pilularia*, 321
Inulin, reactions of, 120; sphere-crystals of, 119
Involucre, 191
Iodine solution, 3, 26, 28
Iris, leaf of, 179; flowers of, 193
Irrigation, 15

Jerusalem Artichoke, 119
Jungermannia, 363, 367
Juniperus, 230

Lacunar tissue, 264
Lamellæ, of leaf of Moss, 341 ; of Mushroom, 256
Lamina, 124, &c.
Laminaria, 406
Lateral buds (*Equisetum*), 336
Lateral roots, 149 ; origin of, 151 ; of Maize, 183 ; of Pine, 248
Latex, reactions of, 118
Laticiferous cells 120 ; vessels, 118
Leaf-scar, 143
Leaf-sheath, Maize, 172 ; of *Equisetum*, 325, 336
Leaf-trace, 57–61 ; of Fern, 290
Leaves, origin of, 79 ; of Dicotyledons, 122 ; bifacial type of, 122 ; isobilateral type of, 134 ; centric type of, 136 ; aquatic type of, 138 ; of Monocotyledons, bifacial type, 172 ; isobilateral type, 179 ; aquatic type, 180 ; of *Selaginella*, 268 ; of *Lycopodium*, 282 ; of Fern, 307 ; of *Pilularia*, 321 ; of Moss, 345 ; of Characeæ, 410 ; fall of leaf, 143
Lemanea, 386, 389
Lenticels, 93
Leontodon, see Dandelion
Lepidium, 491
Leukoplasts, 220
Lichens, 473, &c.
Lignified walls, reactions of, 38
Ligule, 172, 262
Lilac, apex of, 83
Lilium, 176
Lime, sieve tubes of, 116
Limiting layer of Fucus, 395
Linseed, 94
Linum, 94
Lunularia, 361
Lupinus luteus, 228
Lychnis, flower of, 189
Lycoperdon, 461
Lycopodinæ, 261
Lycopodium, 275, &c.
Lysigenetic cavities, 331

Macrosporangia, 205, 255, 271, 326
Macrospores, 205, 271, 323
Macrozoospores, 426
Mahonia, 464
Maize, see *Zea*
Male branches, *Marchantia*, 361, 369
Male inflorescence, Pine, 252
Male Shield Fern, 287 ; see *Aspidium*
Manubrium, 415
Marchantia, 360, &c.
Mares-tail, see *Hippuris*
Marsh Marigold, 188
Marsilea, 320 ; sporocarp of, 324
Measurement of objects, 22
Mechanical strengthening, 140
Medulla, see Pith
Medullary rays, Elm, 89, 98, 102, 105 ; of Pine, 236, 241, 242, 244
Medullary sheath, 89
Megalospora, 478
Meristem, 80, &c.
Mesocarpus, 468
Mesophyll, 127 ; Maize, 174 ; Pine, 245 ; *Lycopodium*, 282 ; Fern, 308
Metzgeria, 367
Micrasterias, 448
Micrometer, 22
Micropyle, 205, 215, 255
Microscope, adjustment of, 16
Microsporangia, 200, 253, 271, 323
Microspores, 200, 253, 271, 323
Microtome, 9
Microzoospores, 426
Middle lamella, 38
Midrib, 124
Mirabilis, fruit of, 217
Monocotyledons, vegetative organs of, 161–186
Morchella, 471
Moss-plant, 341, &c.
Mother-cells, of antherozoids or spermatozoids, 314, 347, 370 ; of pollen, 203 ; of spores, 311, &c.
Mounting objects, 12
Mucilage-cells, Elm, 93
Mucilaginous walls, reactions of, 94
Mucor, 497
Mucorineæ, 497
Multiple epidermis, 139

Musci, 341
Mushroom, 453
Mycelium, of Mushroom, 453; of *Puccinia*, 463; of *Aspergillus*, 485

Navicula, 449
Neck of archegonium, Fern, 316; of Moss, 349
Nectary, 142, 199
Nerves, 124
Neutral conceptacles, 396
Nitella, 34, 469, 414, 416, 417
Node, of Sunflower, 86; of Maize, 165; of *Equisetum*, 329
Nostoc, 451, 475
Nucellus, 205, 256
Nuclear spindle, 85, 445
Nucleolus, 29, 449
Nucleus, 26, 28; division of, 85
Nucule, 476
Nutritive decoction of French Plums, 404

Octants of embryo, 210
Œdogonium, 421
Oil, 222
Oil of cloves, clearing by, 51, 52
Oncidium, aërial roots of, 185
Oogonium, of *Fucus*, 402; of *Chara*, 416; of *Coleochæte*, 420; of *Œdogonium*, 423; of *Vaucheria*, 432; of *Volvox*, 438; of *Pythium*, 494
Oophyte, of Fern, 311; of *Equisetum*, 340; of Moss, 341; of *Marchantia*, 360
Oosphere, see Ovum
Oospore, of *Coleochæte*, 420; of *Œdogonium*, 423; of *Vaucheria*, 432; of *Volvox*, 438; of *Pythium*, 494
Operculum, 342, 352
Oranges, hesperidin of, 120
Oscillatoria, 452
Ostiole, of *Polysiphonia*, 390; of *Fucus*, 401; of *Cetraria*, 480; of *Claviceps*, 483
Ovary, 188, &c.
Ovule, of Angiosperms, 205, &c., of Pine, 255, &c.
Ovuliferous scale, 254
Ovum of Angiosperms, 206; of Pine, 257; of Fern, 316; of Moss, 349; of *Marchantia*, 374; of *Fucus*, 404

Pæonia, 37
Paleæ, 287
Palisade parenchyma, 127
Pandorina, 439
Penicillium, 490
Pappus, 193
Paraffin, 11
Paraphyses, of Mosses, 347; of *Laminaria*, 408; of *Agaricus*, 459; of *Peziza*, 471; of Lichens 477
Parmelia, 473
Pelargonium, colouring of, 198
Pellia, 362, 365, 371, 377
Peltigera, 479
Peperomia, multiple eperdimis of, 139
Perianth, 193
Periblem, 112, 159, 184, &c.
Pericambium, see Pericycle
Pericarp, 216, 217, 225
Pericentral cells, 382
Perichætia, 340
Periclinal walls, 80, &c.
Pericycle, 150, 181, 248, 250, 304
Periderm, 91
Peridium, 466
Perigonium, 340, 347
Perigynium, 376
Peristome, 342, 352, 355
Perithecium, 483, 487
Permanent mounting, 51
Peronospora, 495
Peronosporeæ, 491
Pertusaria, 479
Petals, 187, &c.
Petiole, 122
Peziza, 470
Phæophyceæ, 391
Phallus, 461
Phanerogamæ, 54
Phaseolus, root of, 148
Phellogen, 91

Phloem, of Sunflower, 72; of Elm, 95; of Pine, 242; of Fern, 297, &c.
Phloem-parenchyma, 72, 95, 242
Phloem-sheath, 264, 295, 299, &c.
Phyllocladus, 230
Phyllode of *Acacia*, 134
Phytophthora, 496
Pileus, 455
Piliferous layer, 150, 180
Pillwort, 319
Pilobolus, 499
Pilularia globulifera, 319
Pinnularia, 449
Pinus sylvestris, 229, &c.
Pit-membranes, 214, 238, 382
Pith, of Sunflower, 63, 75; of Elm, 89; of *Rosa* or *Rubus*, 97; of Pine, 232; of Fern, 289
Pits, bordered, 237, 240, &c.
Pitted vessels, 74, 103
Plasmolysis, 31
Plerome, 112, 159, 184, 211
Pleurococcus, 440
Plugs in pollen-tubes, 208
Plumule, 211, 215
Poinsettia, glands of, 142
Pollen, 201, 253
Pollen-grains, germination of, 202
Pollen-mother-cells, 203
Pollen-sacs, 200, 253
Pollen-tube, 202, 207, 256
Pollinodium, 488
Polyembryony, 258
Polyporus, 460
Polysiphonia, 379
Polytrichum, 341
Pores of *Sphagnum*, 258
Potamogeton, endosperm of, 213
Potash solution, 2, 29; clearing by, 49
Potato, crystalloids of, 225; starch of, 44
Preservation of material, 4
Primary root, 148
Primary tapetal layer, 203
Primordial utricle, 43
Primrose (*Primula*), flower of, 190
Procambium, 81
Proembryo, 417
Promycelium, 463
Prothallus of Fern, 30, 311
Protonema of Moss, 356
Protophloem, 295
Protoplasm, 26, 28; continuity of, 213; movements of, 33
Protoxylem, 103, 182, 233, 297, 299, &c.
Prunus, glands of, 142
Prunus Padus, flowers of, 189
Pseudopodium, 359
Pteridophyta, 261, &c.
Pteris, stem, 298
Ptilota, 385
Puccinia, 462, &c.
Pulvinus, 123
Punctum vegetationis, see Apex.
Pyrenoids, of *Spirogyra*, 444; of *Closterium*, 447
Pyrenomycetes, 481
Pythium, 491

Radicle, 211, 215, &c.
Ramenta, 287
Ranunculus, flower of, 187
Raphe, 205
Raphides, 178
Ray-florets, 191
Razor, 8
Reagents, 2, and Appendix A.
Reduced vascular bundles, of *Hippuris*, 110; of *Elodea*, 171
Reproductive organs, of Angiosperms, 187; of Gymnosperms, 252
Resin, reactions of, 65
Resin-passages, 65, 233, &c.
Resting conidia, 493
Rhizines, 473
Rhizogenic cells, 304
Rhizoids, 341, 361, &c.
Rhizome, of *Pteris*, 298; of *Pilularia*, 319
Rhizophores, 262, 267
Rhododendron, style and stigma of, 208
Riccia, 62
Ricinus, aleurone-grains of, 223; endosperm of, 216; seed of, 73; seedlings of, 226; interfascicular cambium of, 79

Ring of sporangium, 310
Ripe seed (Pine), 258
Robinia, thyloses of, 75
Root of Dicotyledons, 148; of Monocotyledons, 180; of Pine, 247; of *Taxus*, 250; of *Selaginella*, 268; of *Lycopodium*, 283; of Fern, 303; of *Equisetum*, 337
Root, apex of, 157, 183, 251, 306, &c.
Root-cap, 159, 185, 251, 307, &c.
Root-hairs, 33, 150, 182, 250, &c.
Root, secondary thickening of, 152, 249
Rosa, pith of, 97
Rose Campion, flower of, 189
Rotation of protoplasm, 33, 414
Rubus, pith of, 97
Rudimentary gynœcium and stamens, 189
Ruscus, 37
Russula, 500
Rust of Wheat, 462
Rye, Ergot of, 481

Salt solution, 3, 26, 29, 31
Saxifraga, chalk-glands of, 142
Scalariform tracheides, 267, 297
Scales of bulb, Hyacinth, 167
Scale-hairs, 146
Scale-leaves, Pine, 229
Scape of Hyacinth, 167
Schulze's solution, see Chlor-zinc-iodine
Schulze's macerating fluid, 104
Scilla (Blue-bell), flower of, 193
Sclerenchyma, of Sunflower, 67, 72; of Elm, 95; of *Cucurbita*, 113; distribution of, 140; of Maize, 161; in leaf, 173; of *Lycopodium*, 276; of Ferns, 289, 293, 296, 298; of Moss, 343
Sclerotium, 481
Scutellum, 227
Secondary thickening, of stem, 97, 169, 230; of root, 153, 248; of Monocotyledons, 169; of *Laminaria*, 407
Sedum, leaf of, 136
Seed coat, 215
Seed, of Dicotyledons, 215; of Pine, 258
Segmental cells, 302, 307
Selaginella, 261, &c.
Seta, 342, 350, &c.
Sexual organs, of Ferns, 313; of Mosses, 347; of Liverworts, 369, &c.; of Characeæ, 410; of Florideæ, 387; of *Fucus*, 301; of *Vaucheria*, 431; of *Volvox*, 439; Lichens, 479; of *Pythium*, 494
Sieve-plates, see sieve-tubes
Sieve-tubes, Sunflower, 68, 72; of Elm, 102; of *Cucurbita*, 113; of *Tilia*, 116; of Maize, 164; of Pine, 236, 242; of Ferns, 295, &c., of *Equisetum*, 384
Silica-skeletons, 90, 333
Silicified cell-walls, 90
Siphoneæ, 429
Soft-bast, see Phloem
Soredia, 476
Sori, 309
Spawn of Mushroom, 453
Special-mother-cells, 204
Spermatia, 480, 388
Spermatozoids of Fern, 314; of Moss, 347; of *Fucus*, 403; of *Chara*, 415; of *Vaucheria*, 432, &c.
Spermogonia, 467, 479
Sphacelia, 483
Sphagnum, 357
Sphere-crystals of inulin, 120
Spindle-threads, 85
Spiral vessels, of Sunflower, 74; of Elm, 103
Spirogyra, 27, 442
Spongy parenchyma, 127, 132, &c.
Sporangium of *Selaginella*, 270; of *Lycopodium*, 284; of Fern, 309; of *Pilularia*, 322; of *Equisetum*, 338; of *Laminaria*, 408; of *Mucor*, 498; of *Sporodinia*, 501
Spores, of *Selaginella*, 271; of *Lycopodium*, 284; of Fern, 309; of *Equisetum*, 339; of Moss, 353; of *Marchantia*, 376; of Mushroom, 459; of *Peziza*, 471; of Lichens, 477; of *Claviceps*, 483; of *Mucor*, 498

Spore-mother-cells, 272, 286, 311, 352, &c.
Spore-sac (Moss), 352
Sporidium, 464
Sporocarp, *Pilularia*, 322
Sporodinia, 500
Sporogonium, of Moss, 350 ; of *Sphagnum*, 359 ; of *Marchantia*, 361, 376 ; of *Anthoceros*, 378
Sporophyte, of *Selaginella*, 260 ; of *Lycopodium*, 275 ; of Fern, 287 ; of *Equisetum*, 325
Spruce Fir, 229
Spurious tissue, 457
Stachys, bundle system of, 57, 59
Staining, 48
Stamen, 188, 200
Starch, structure and reactions of, 44 ; optical properties of, 47
Starch-forming corpuscles, 220
Stellaria, flower of, 188 ; pollen-tubes of, 206
Stem, herbaceous Dicotyledon, 55 ; arboreous Dicotyledon, 88 ; aquatic Dicotyledon, 110 ; Monocotyledons, 161 ; of Pine, 232 ; of *Lycopodium*, 275 ; of *Selaginella*, 261 ; of Fern, 292 ; of *Equisetum*, 327 ; of Moss, 342 ; of *Sphagnum*, 357
Sterigmata, 459, 464, 467, 486
Stigma, 207
Stinging hairs, 145
Stipe, 455, 456
Stichwort, flower of, 188
Stomata, 127, 133, &c. ; of Maize, 172 ; of Hyacinth, development of, 177 : of leaf of Maize, 173 ; of Hyacinth, 175 ; of *Lilium*, 176 ; closing of, 176 ; of Pine, 246 ; of *Selaginella*, 268 ; of *Lycopodium*, 282 ; of Fern, 309 ; of *Equisetum*, 333 ; of Moss, 352 ; of *Marchantia*, 363
Stratification of cell-walls, 40
Striation of cell-walls, 40
Stroma, 482
Strychnos, 214
Style, 207
Subsidiary cells, 137, 175, 333
Sub-hymeneal layer, 459
Sugar, 221
Sunflower, see *Helianthus*
Suppression, 189
Suspensor, 210, 211, 257 ; of *Sporodinia*, 501
Suture of carpel, 205
Swarm spores, 420, 423
Sympodium, 360, 393
Synergidæ, 206
Syngenesious stamens, 192
Syzygites, 500

Tapetum, 203, 206, 272, 285, 311
Taraxacum, 118, 193
Taxus, leaves of, 247 ; root of, 250 ; reproductive organs of, 259
Teleutospores, 463, 469
Tetraspores, 387
Thallophyta, 379
Theca, 342, 352
Thuja, leaves of, 230
Thyloses, 75
Tilia, sieve-tubes of, 116
Tomata, chromoplasts of, 199
Torula, 500
Trabeculæ, 264
Trabecular tissue, 397
Tracheides, of Pine, 237, &c. ; of *Selaginella*, 267 ; of Fern, 297, 300 ; of *Equisetum*, 331
Tradescantia, 34, 84
Trama, 459
Transfusion tissue, 247
Trianea, 33
Trichogyne, 389, 480
Tropæolum, 199
Tubers of *Equisetum*, 332
Tulip, 198
Turpentine, 52

Ulmus, see Elm
Ulothrix, 424
Underground stem, *Equisetum*, 326
Uredospores, 468
Usnea, 475

Vacuole, 26
Vacuolization, 83
Vaginula,

Vallisneria, 34
Vanda, 185
Vascular bundles, systems of, 56–61 ; of Sunflower, 56 ; of Maize, 166 ; of Ferns, 289
Vascular network, in stem of Ferns, 289
Vaucheria, 429
Velamen, 185
Velum partiale, 455
Ventral canal-cell, 315, 375
Vessels, 73, &c. ; laticiferous, 118
Viburnum, glands of, 142
Vitis, bark of, 101
Volvocineæ, 437
Volvox, 437

Wallflower, leaf of, 127
Walnut, seed of, 216
Water of imbibition, 36
Water stomata, 141
White Rust, 495
Winter-spores, 463, 469
Wood, see Xylem
Wood-fibres, 96
Wrack, 391

Xylem, of Sunflower, 73 ; of Elm, 96, &c. ; of Pine, 133, &c.
Xylem fibres, 96, &c.
Xylem parenchyma, 96, &c.

Yucca, stem of, 169

Zea, fruit of, 218 ; germination of, 227 ; stem of, 161 ; apex of, 166 ; leaf of, 172 ; root of, 180 ; apex of root, 183
Zoogonidia, 433
Zoosporangia, 494
Zoospores, of *Ulothrix*, 426 ; of *Pythium*, 494 ; of *Cystopus*, 496
Zygnema, 442
Zygospores, of *Ulothrix*, 426 ; of *Sporodinia*, 500, 501
Zygote, of *Closterium*, 448 ; of *Spirogyra*, 446 ; of *Sporodinia*, 501

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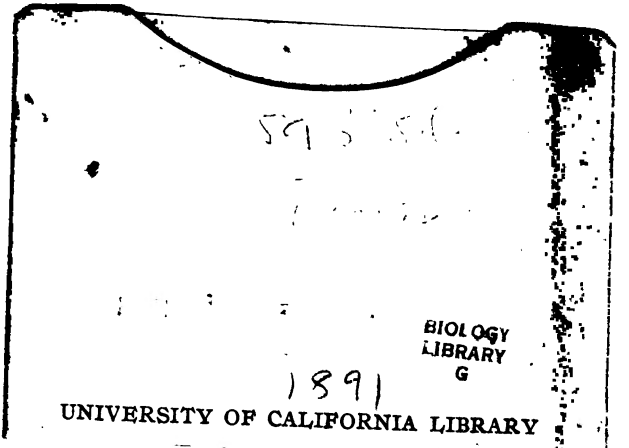
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